VOLUME LXVI NUMBER 2

THE
BOTANICAL GAZETTE

AUGUST 1918

DETERMINATION OF WILTING

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 241

ARTHUR L. BAKKE

(WITH FIVE FIGURES)

The status of the question of permanent wilting in plants, as described by BRIGGS and SHANTZ (**5, 6, 7, 8**), CALDWELL (**11**), SHIVE and LIVINGSTON (**37**), and ALWAY (**1**), centers about the determination made by BRIGGS and SHANTZ that a plant is regarded as having attained a condition of permanent wilting when it does not recover its turgidity in a period of 24 hours when surrounded by air saturated with water vapor. The method of employing standardized hygrometric paper (**2, 3, 4, 28, 30, 38, 40, 42**) in the measurement of the transpiring power in plants consists in ascertaining the power of a leaf to give off water and comparing this with the power represented by a saturated blotting paper surface at the same time. This is then a measure in both cases of the resistance to the passage of water. The conditions which affect such measurements are internal, but these internal factors are dependent upon external factors. It is obvious, therefore, that data derived will be more or less of a resultant complex of all the forces which have been operative during the history of the plant.

The method in principle is the same as has previously been used in investigations upon the foliar transpiring power of plants. In the present studies filter paper circles (Munktell's Swedish

no. 00—11 cm.)[1] are impregnated with 3 per cent solution of cobalt chloride and are later cut into small squares. Just before using, these squares are heated over a bicycle lamp, or on a granite pie-plate suspended by a clamp over the flame of an alcohol lamp, until they become blue. One of these squares is placed between the jaws of a "transpiration clip," and as quickly as possible applied to either the upper or lower surface of a leaf. The time required to change the paper square from blue to pink is determined in seconds. The time which it takes to change a similar piece of cobalt paper from blue to pink when placed over a moist blotting paper surface blanketed by a millimeter of air (**2, 4, 28, 30, 34, 42**) is recorded. The water apparatus is the same as used by BAKKE and LIVINGSTON (**4**). TRELEASE and LIVINGSTON (**42**) have developed the relations of the temperature to vapor tension as first shown by BAKKE (**2**). These authors have presented a formula whereby the time interval may be ascertained on knowing the temperature. LIVINGSTON and SHREVE (**30**) have recently improved and modified this method. The principal improvement is in the adoption of permanent color standards. Instead of the simple square of cobalt chloride paper, a composite slip is employed consisting of a small piece of the hygrometric paper in juxtaposition with two slips having permanent color standards. These provide both an initial and an end point for the color change. For use in the laboratory they advocate and describe a simpler form of standard water evaporating apparatus. These modifications were not used in this study.

The possibilities of using the original method of standardized hygrometric paper in determining the extent of wilting and the permanent wilting point was first suggested to me by its author, B. E. LIVINGSTON, at the Desert Laboratory of the Carnegie Institution during the summer of 1913. In 1914 the writer (**3**), working at the Desert Laboratory, performed a series of measurements upon sunflower plants lifted from the soil and later brought into the laboratory to wilt. The results of this series of tests show that

[1] LIVINGSTON and SHREVE in a more recent publication (Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant World **19**: 287–309. 1916) have recommended Whatman's filter no. 30 (11 cms.) circles as being superior to the Swedish paper.

wilting occurs at a definite point and that permanent wilting represents the most intense wilting possible, without serious rupture of the water columns of the plant. These studies have been amplified in the present investigation. The experimentation involved in the present study was performed in the greenhouse of the University of Chicago during the summers of 1915 and 1916. The large Russian variety of the common sunflower (*Helianthus annuus*) was used, the seed being from W. W. BARNARD of Chicago. The experiments involving the porometer were performed in the laboratory of Plant Physiology of Iowa State College. The plants were the same variety, but seed was secured from the Iowa Seed Company of Des Moines, Iowa.

Series of 1915

METHOD

The seeds used in the tests for 1915 were planted in sheet iron containers 6×6 inches, on June 31. Germination was forced by placing the containers in a warm house. When the cotyledons had made their appearance, the seedlings were thinned out so that only 3 remained. The cultures were then removed to a cooler place, where the plants were allowed to grow until they were approximately 6 weeks old and about 40 cm. high. The soil used in this series consisted of 4 parts of compost and 1 part sand. The water-holding capacity was calculated to be 47 per cent. The plants remained in the same containers throughout the entire period of the experiment. They were watered from time to time until the morning of July 13, when they were heavily watered, and after that no more water was added until the morning of July 16, when the plants were lightly watered and the soil surface covered with plasticine. Two plants were used as checks in testing out wilting by the BRIGGS and SHANTZ method.

The values for the indices of foliar transpiring power were obtained according to the original Livingston method; the standard water apparatus was the same as described by BAKKE and LIVINGSTON. Throughout the series, cobalt paper squares made from Munktell's Swedish no. 00 filter paper were used. As the work was carried on in the greenhouse, the usual bicycle lamp

for lighting was replaced by electric light. The cobalt paper squares were warmed upon a granite pie-plate, which was adjusted by a clamp over an alcohol flame, so that the paper squares were heated to a temperature sufficient to give them the blue color.

EXPERIMENTATION

The readings for the 1915 series, begun on August 16, were usually made between the 10th and 11th hours and again between the 20th and 21st hours. Two plants were used for the foliar transpiring power tests; two additional plants were used for the wilting determinations according to the method of BRIGGS and SHANTZ. Evaporation was determined at the same time by a standardized Livingston form of cylindrical atmometer. The readings as recorded in table I show the maximum foliar transpiring power as occurring about the 11th hour, while the minimum usually occurs after sunset. Wherever possible, leaves of different ages were used and were numbered and tagged Ia_1, Ia_2, Ia_3, Ib_1, Ib_2, etc., the highest number representing the youngest leaf. In this way the same leaf could be used throughout.

The average results of the foliar transpiring power indices, as represented graphically (fig. 1), show a general decline from August 16 to August 20. The maximum index reached on August 17 possesses a value of 0.89. This index is almost the same as the one obtained earlier by BAKKE and LIVINGSTON. Although the plants were watered a little on the day the experiment was begun, they must have given off considerable water during the previous 3-day interval. That the soil moisture content has an appreciable effect upon foliar transpiring power has been proven previously, and from the nature of transpiration it is self-evident. The *Helianthus* plants of BAKKE and LIVINGSTON were growing in a place where the soil moisture was less than would be regarded as optimum. In all probability the two sets of *Helianthus* plants were grown in soil having practically the same amount of moisture. The soil moisture content in both series was below the amount necessary for the production of the greatest growth.

For the first half of the series the highest transpiring power occurs during the day, while the lowest transpiring power values

are at night. The average day values are accordingly 0.72, 0.92, 0.74, 0.38, 0.26, 0.19, 0.32 for one set (I*a*); for the other (I*b*), 0.61, 0.89, 0.76, 0.30, 0.30, 0.39, 0.42. The average night values for the first series are 0.29, 0.34, 0.24, 0.19, 0.25, 0.44, 0.69; for the second series, 0.31, 0.39, 0.23, 0.16, 0.50, 0.45, 0.61. The results obtained by calculating the ratio of the respective day and night values are rather uniform. For August 16 the average ratio is 2.4; for August 17, 2.7; the remaining values for I*a* are 3.1, 2.0, 1.0, 0.43, 0.46. The corresponding respective values for series I*b* are 2.0, 2.3, 3.3, 1.9, 0.77, 0.87, 0.61. For the first two days, August 16 and 17, the probable normal ratio is between 2 and 3. On the following day there is a slight increase, and after that there is a decrease. Whether the rise in the ratio on the third day presents a normal situation or not cannot at present be stated; at any rate the value is not far from 3. The decrease in foliar transpiring power after August 19 and the resulting decrease in the ratio do not show any definite mathematical relation. For a plant growing in a normal environment, a rise in evaporation will give an increase in transpiring power, but on August 22 there is a high evaporation, a low foliar transpiring power, and a lower day value than night value. Such a status must be looked upon as abnormal for growing plants. Beginning with August 21 there is a rapid ascent.

Considerable agreement is present between the graphs in this series and the one for *Helianthus* (3), where the plants were lifted from the soil. There is a decrease in the foliar transpiring power to a point where there is more or less of a balance, and then again where there is an increase. The time element in the present series is extended over a longer period, and as a result variations which might be masked in the series of short duration would be present.

The rupture of the water columns of the plants of the 1915 series is as definite as that presented for the plants lifted from the soil in southern Arizona. The outstanding feature of the curve is the very marked rise on August 20. Upon examination of the rate of evaporation, it will be at once evident that the evaporating power of the air was very low throughout. Two plants of this

TABLE I

INDICES OF FOLIAR TRANSPIRING POWER FOR 3 DIFFERENT LEAVES OF TWO *Helianthus* PLANTS DURING PROGRESSIVE MARCH OF WILTING FROM AUGUST 16–22, 1915 (MAXIMA IN BOLD FACED TYPE, MINIMA IN ITALICS)

LEAF NUMBER	DAY AND HOUR OF OBSERVATION	INDEX OF TRANSPIRING POWER			EVAPORATION FROM STANDARDIZED ATMOMETER, CC. PER HOUR	DAY AND HOUR OF OBSERVATION	INDEX OF TRANSPIRING POWER			EVAPORATION FROM STANDARDIZED ATMOMETER, CC. PER HOUR	RELATION OF DAY TO NIGHT
		Lower	Upper	Entire			Lower	Upper	Entire		
	August 16					August 16					
Ia_1	11:00	0.64	0.49	0.56		20:00	0.27	0.18	0.23	0.45	2.4
Ia_2		0.94	0.52	0.73			0.36	0.23	0.30		2.4
Ia_3		0.98	0.74	0.86			0.41	0.27	0.34		2.5
		Average 0.85	0.58	0.72			0.35	0.23	0.29		2.4
Ib_1	11:00	0.74	0.36	0.55			0.41	0.19	0.30		1.8
Ib_2		0.94	0.39	0.67			0.40	0.22	0.31		2.2
		Average 0.84	0.38	0.61			0.41	0.21	0.31		2.0
	August 17					August 17					
Ia_1	10:00	0.89	0.80	0.85	0.40	21:00	0.39	0.20	0.30	0.17	2.8
Ia_2		0.94	0.94	0.94			0.42	0.24	0.33		2.8
Ia_3		0.97	0.97	0.97			0.48	0.32	0.40		2.4
		Average **0.93**	**0.90**	**0.92**			0.43	0.25	0.34		2.7
Ib_1		0.97	0.64	0.80			0.52	0.24	0.38		2.1
Ib_2		0.97	0.97	0.97			0.51	0.29	0.40		2.4
		Average **0.97**	**0.81**	**0.89**			0.52	0.27	0.39		2.3
	August 18					August 18					
Ia_1		0.74	0.16	0.45	0.18	20:00	0.29	0.14	0.22	0.64	2.0
Ia_2		0.84	0.84	0.84			0.32	0.17	0.24		3.5
Ia_3		0.97	0.89	0.93			0.34	0.16	0.25		3.7
		Average 0.85	0.63	0.74			0.32	*0.15*	0.24		3.1
Ib_1		0.67	0.62	0.65			0.32	0.18	0.25		2.6
Ib_2		0.91	0.82	0.86			0.26	0.17	0.21		4.1
		Average 0.79	0.72	0.76			0.29	0.18	0.23		3.3
	August 19					August 19					
Ia_1	10:00	0.51	0.35	0.43	0.06	20:00	0.21	0.16	0.18	0.67	2.4
Ia_2		0.38	0.32	0.35			0.24	0.21	0.22		1.6
Ia_3		0.40	0.35	0.37			0.21	0.13	0.17		2.1
		Average 0.43	0.34	0.38			*0.22*	0.17	*0.19*		2.0
Ib_1		0.37	*0.22*	0.30			0.20	0.13	0.17		1.8
Ib_2		0.37	0.23	0.30			0.18	0.13	0.15		2.0
		Average 0.37	*0.23*	*0.30*			*0.19*	*0.13*	*0.16*		1.9

	August 20					August 20					
Ia_1	11:00		0.31	0.18	0.24	0.08	21:00	0.27	0.27	0.27	0.27	0.89*
Ia_2			0.30	0.21	0.26			0.27	0.19	0.23		1.10
Ia_3			0.31	0.24	0.27			0.25	0.24	0.25		1.10
		Average	0.30	0.21	0.26			0.26	0.23	0.25		1.00
Ib_1			0.33	0.24	0.28			0.38	0.32	0.35		0.80
Ib_2			0.30	0.33	0.32			0.44	0.41	0.43		0.75
		Average	*0.32*	0.29	*0.30*			0.75	0.69	0.72		0.77*
								0.52	0.47	0.50		0.91
	August 21						August 21					
Ia_1	11:00		0.20	0.15	0.18	0.06	21:00	0.83	0.29	0.56	0.24	0.34
Ia_2			0.26	0.17	0.22			0.60	0.37	0.49		0.45
Ia_3			0.23	0.12	0.18			0.28	0.24	0.26		0.69
		Average	*0.23*	*0.15*	*0.19*			0.57	0.33	0.44		0.43
Ib_1			0.39	0.17	0.28			0.78	0.43	0.61		0.46
Ib_2			0.31	0.17	0.24			0.59	0.42	0.51		0.47
Ib_3			0.67	0.62	0.64			0.27	0.27	0.27		2.30
		Average	0.46	0.32	0.39			0.54	0.37	0.46		0.87
	August 22						August 22					
Ia_1	11:00		0.69	0.29	0.49	0.04	21:00	0.70	0.60	0.65	0.66	0.75
Ia_2			0.34	0.21	0.27			0.73	0.73	0.73		0.37
Ia_3			0.24	0.15	0.19			0.73	0.63	0.68		0.30
		Average	0.42	0.22	0.32			**0.72**	**0.65**	**0.69**		0.46
Ib_1			0.62	0.50	0.56			0.80	0.83	0.82		0.68
Ib_2			0.48	0.29	0.38			0.85	0.82	0.83		0.46
Ib_3			0.34	0.28	0.31			0.43	0.42	0.42		0.74
		Average	0.48	0.36	0.42			**0.69**	**0.69**	**0.69**		0.61

* Since the experiment has been in progress a new leaf has become of sufficient size so that it was large enough for the application of the clip. For August 21 then the index 0.72 is much higher than any of the others. This will naturally increase the average. In computing the average for the index, this last number was not used; 0.77 is the average for leaves Ib_1 and Ib_2.

same series were used for the determination of wilting according to the method of BRIGGS and SHANTZ. The results are given

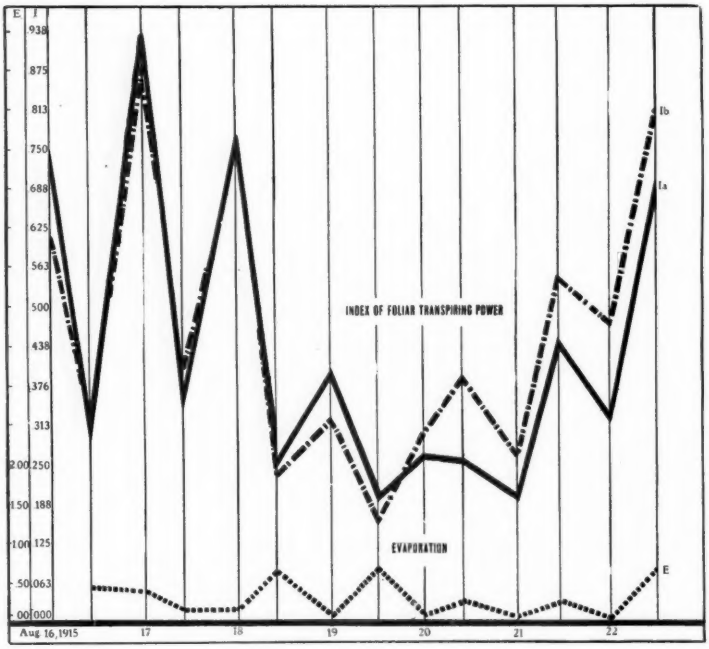


FIG. 1

in table II; those obtained giving the residual moisture at the time of wilting agree rather closely for the determinations made according to the two methods. In the method of BRIGGS and

TABLE II

Method	1	2	Average
Briggs and Shantz.....	10.38	7.84	9.11
Hygrometric paper.....	8.20	8.34	8.27

SHANTZ there is a greater variation than is found to be present where the hygrometric paper is used.

The breaking point occurring on August 21 is not far from the normal minimum value of the daily march of foliar transpiring power. From previous work upon the march of foliar transpiring power, there is more or less of a definite maximum (usually during the day) as well as a definite minimum (usually during the night). It seems that, in all probability, the minimum in the foliar transpiring power indicates approximately the greatest resistance to transpirational water loss. If the water content of the soil coupled with the evaporating power of the air is of such magnitude as to increase the resistance to the passage of water, so that the day maximum has a value as low or lower than the diurnal minimum (at night), the plant is then in a critical condition; at least this has been found to be true for *Helianthus*. For the entire leaf surface the transpiring power ratios at night are as follows: (1) I*a*, −0.23, 0.30, 0.34; (2) I*b*, −0.30, 0.31. On August 20 the respective values are 0.24, 0.26, 0.27 for Ia_2, and 0.28 and 0.32 for Ib_2. The average ratio for the first is 2.2 and the average ratio for the test on August 20 is 0.91. On August 21 the ratio is less; on the following day it is a little higher.

The entire situation as here brought forward centers about the amount of moisture present in the soil during the march of wilting when the index of transpiring power ratio of day to night comes to be represented by unity or less. The duration of this ratio may be an important factor in obtaining data that will give information on the relative drought resistance of plants.

Series of 1916

METHOD

The method of procedure in the experimentation for 1916 was much the same as for the previous season. The sheet iron containers were a little deeper (7 inches instead of 6). The soil mixture was lighter than before, containing 1 part of clean pure sand mixed with 3 parts of rich garden soil, and the variety the same as before (Mammoth Russian). The seeds were planted on June 24, and on July 1 the seedlings were 5 cm. high and were then transplanted. Three plants were set deeply in the soil. The cultures

were then placed in the greenhouse and were watered from time to time. A Livingston standard atmometer of the cylindrical form was set up in close proximity to measure evaporation. Readings were taken of the atmometer whenever a reading was made of the transpiring power. On July 19 the plants were thoroughly watered and were lightly watered again on July 20. On July 21 the containers were sealed over with plasticine preparatory to making hourly readings of the foliar transpiring power for a period of twenty-four consecutive hours. For the measurements upon the index of foliar transpiring power the same apparatus as employed before was used. On the 18th hour of July 22 the last reading was made for the daily march of foliar transpiring power. Beginning July 24, readings were taken three times during the day: (1) at approximately 10:00 A.M., (2) at the 14th or 15th hour, and (3) at some time during the night. The times chosen really represent the three important periods during the daily march, for the first one gives this value at a time when the transpiring power is near its maximum, the second when evaporation is at its maximum, and the third when the index of transpiring power has its lowest value. The leaves were tagged as before so that the same leaves were used throughout.

The soil surface of several additional plants was coated over with plasticine to serve as a comparison or check for the plants used for the determination of foliar transpiring power. In applying the cobalt paper squares from day to day, it became easy to judge the condition of the plant. When plants presenting a physical state such as was in evidence for leaf Ia_2 on August 3, and for leaf Ib_2 on August 7, were placed in a moist chamber, they failed to recover. It was then deemed unnecessary to test further. At the time of the beginning of the experiment plant I*a* was 25 cm. high, while plant I*b* was 28 cm. high.

INDICES OF FOLIAR TRANSPIRING POWER

In using the method of standardized hygrometric paper for the determination of the indices of foliar transpiring power, two separate plants were used. The method of numbering the leaves was the same as for the 1915 series. From plant I*a* two leaves

were chosen, Ia_1 having the dimensions 5×8 cm., and Ia_2, 4×6 cm.; from plants I*b* two leaves were chosen, Ib_1, 7×9 cm., and Ib_2, 3×4 cm. Whenever a new leaf became sufficiently large for the application of the clip, approximately 3×4 cm., it was included with the others. The readings were begun on the 18th hour of July 21 and continued at hourly intervals for 24 hours. Readings were taken at the same time from a standardized Livingston cylindrical form of atmometer. The results for plant I*a* are given in table III.

This series shows that the march of the foliar transpiring power is the same as has previously been pointed out (**2, 4, 28, 40**), in that the maximum transpiring power is attained at a time previous to the greatest evaporation. The highest index occurs usually at the 10th and 11th hours, while the evaporation maximum occurs usually on the 14th hour. On account of the larger number of readings it is to be expected that the graphical representation (fig. 2) will show less abruptness than has formerly been presented.

Recalling that the leaf represented by Ia_1 is older than Ia_2, it is plain that the index of foliar transpiring power is higher for the younger leaf almost entirely throughout the 24-hour period. The maximum for Ia_1 is at the 11th hour, when it is 0.93. This value is again in evidence 2 hours later. For Ia_2 the highest value is at the 10th hour, when the transpiring power value is 1.00. This same value is again reached at the 12th hour. It is evident that the younger leaf Ia_2 reaches its maximum at an earlier period than the older leaf Ia_1. This feature substantiates similar conclusions reached by BAKKE and LIVINGSTON. Another important feature in connection with the graph showing the march of foliar transpiring power is the sudden drop for both leaves. The lowest point (0.59 for Ia_1 and 0.67 for Ia_2) occurs on the 14th hour. At the 15th hour the index values are respectively 0.91 and 0.93, while the corresponding values at the 13th hour are 0.93 and 0.83. Although the drop is the feature in the afternoon readings, the recovery occurring at the 15th hour is always below that of the forenoon maximum. In the present case there is not much difference, being 0.91 for Ia_1; for the younger leaves there is a greater variation, being 0.93 at 15:00 o'clock and 1.00 for the 10:00 and 13:00 o'clock readings. At the 14th hour the average reading for the

TABLE III

DATA FOR MARCH OF INDICES OF FOLIAR TRANSPIRING POWER FOR *Helianthus* PLANT 1*a* (MAXIMA IN BOLD FACED TYPE, MINIMA IN ITALICS)

LEAF NUMBER	TIME OF OBSERVATION	TIME OF COLOR CHANGE IN SECONDS		INDEX OF TRANSPIRING POWER			EVAPORATION FROM STANDARDIZED ATMOMETER, CC. PER HOUR
		Lower surface	Upper surface	Lower surface	Upper surface	Entire leaf	
	July 21						
Ia_1	18:15	31	52	0.77	0.46	0.62	
Ia_2	18:20	24	46	**1.00**	0.52	0.76	
Ia_1	19:10	42	65	0.68	0.44	0.56	0.22
Ia_2	19:15	38	55	0.75	0.52	0.64	
Ia_1	20:15	46	59	0.62	0.49	0.56	0.15
Ia_2	20:20	40	54	0.73	0.54	0.64	
Ia_1	21:10	45	72	0.64	0.40	0.52	0.16
Ia_2	21:10	40	50	0.73	0.58	0.66	
Ia_1	22:10	51	77	0.61	0.40	0.51	*0.09*
Ia_2	22:15	40	65	0.78	0.48	0.63	
Ia_1	23:10	56	74	*0.57*	0.43	*0.50*	0.15
Ia_2	23:10	45	69	0.71	0.46	0.59	
	July 22						
Ia_1	24:10	50	90	0.66	*0.37*	0.52	0.18
Ia_2	24:15	42	77	0.79	0.43	0.62	
Ia_1	1:10	48	88	0.71	0.38	0.55	0.12
Ia_2	1:15	42	74	0.81	0.46	0.64	
Ia_1	2:10	57	90	0.63	0.40	0.52	0.15
Ia_2	2:15	49	85	0.74	*0.42*	*0.58*	
Ia_1	3:10	55	85	0.65	0.42	0.54	0.15
Ia_2	3:15	50	75	0.72	0.48	0.60	
Ia_1	4:10	50	85	0.72	0.42	0.57	0.15
Ia_2	4:15	46	78	0.78	0.46	0.62	
Ia_1	5:10	50	62	0.72	0.58	0.65	0.15
Ia_2	5:15	45	57	0.80	0.63	0.72	
Ia_1	6:10	39	50	0.87	0.68	0.78	0.15
Ia_2	6:15	36	50	0.94	0.68	0.81	
Ia_1	7:10	36	42	0.81	0.69	0.75	0.15
Ia_2	1:10	36	37	0.81	0.78	0.80	
Ia_1	8:15	30	42	0.77	0.55	0.66	0.15
Ia_2	8:20	28	32	0.82	0.72	0.77	
Ia_1	9:05	24	24	0.88	0.88	0.88	0.22
Ia_2	9:05	22	22	0.95	0.95	0.95	
Ia_1	10:05	22	24	**0.95**	0.88	0.92	0.58
Ia_2	10:05	21	21	**1.00**	*1.00*	**1.00**	
Ia_1	11:00	21	20	0.91	**0.95**	0.93	.....
Ia_2	11:00	20	20	0.95	0.95	0.95	
Ia_1	12:00	21	21	0.86	0.86	0.86	.....
Ia_2	12:00	18	18	*1.00*	*1.00*	**1.00**	
Ia_1	13:10	16	16	0.93	0.93	0.93	1.0
Ia_2	13:10	18	18	0.83	0.83	0.83	
Ia_1	14:10	17	17	0.59	0.59	0.59	1.2
Ia_2	14:15	15	15	0.67	0.67	0.67	
Ia_1	15:10	15	16	0.93	0.88	0.91	1.2
Ia_2	15:15	15	15	0.93	0.93	0.93	
Ia_1	16:15	17	18	0.88	0.88	0.88	**1.6**
Ia_2	16:20	21	21	0.71	0.71	0.71	
Ia_1	17:00	19	19	**0.95**	**0.95**	**0.95**	1.0
Ia_2	17:00	18	20	**1.00**	0.90	0.95	
Ia_1	18:00	25	35	0.80	0.57	0.69	1.0
Ia_2	18:05	20	25	**1.00**	0.80	0.90	

older leaves (Ia_1) is 0.59 and for the younger leaves is 0.67. The differences then in the order given are 0.33 and 0.34. The respective values on the 15th hour are 0.91 and 0.93. These give recovery value differences of 0.32 and 0.26. The drop in the afternoon reading is not a new thing, either in foliar transpiring power or in transpiration. No doubt this great resistance to the

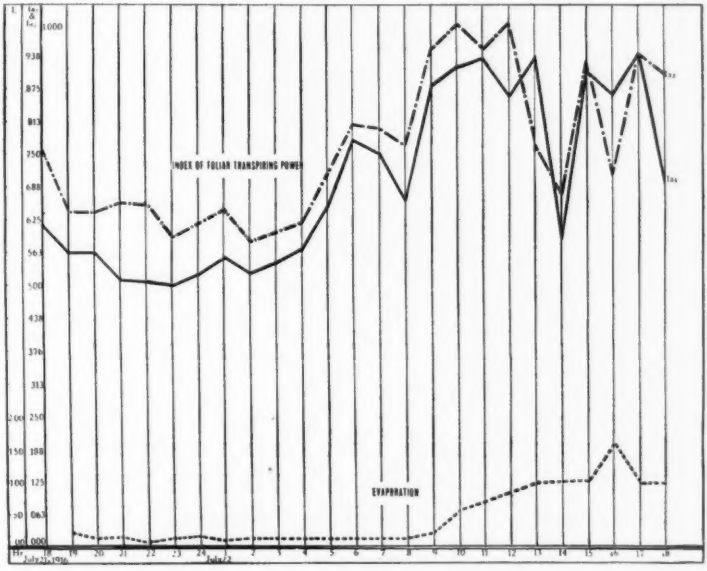


FIG. 2

passage of water is a condition of incipient drying. It may be that at this period, usually present at about the time of greatest evaporation, there is a lack of water, not only in the cells of the leaf, but also in the vessels themselves. SHREVE (**40**) has submitted evidence, at least theoretical, showing that variations in the transpiring power are due to variations in the water-holding capacity of the internal tissue. Using the DIXON (**16, 17, 18**) conception of continuous columns, as well as the results of the experiments of RENNER (**34, 35, 36**) upon transpiration, there is doubtless a greater tension present upon the water columns. If this is related to

absorption and incipient drying, an additional force must be present in order to cause the water to be pulled into the cells to a greater degree than before. If this interpretation is correct, the older leaves (on account of their closer proximity to the absorption center) should show a more complete revival. This speculation would necessarily be based upon the readings of the secondary maxima. The comparative values become evident, for I*a* has a maximum of 0.93, falls to 0.59, and subsequently returns to 0.91; for Ia_2 the maximum is 1.00 and comes back from 0.97 to 0.86. This fall and subsequent rise are independent of the evaporation rate.

The march of the foliar transpiring power is more or less definite. This is especially true as it bears upon maximum and minimum values. For I*a* the maximum value occurs on the 11th hour with an index of 0.95, while the minimum value 0.50 is on the 23d hour. The ratio between maximum and minimum is 1.9. For Ia_2 the maximum value, 1.00, is in evidence on the 10th hour, while the reading 0.58 on the 2d hour of July 22 gives the minimum value. The ratio in the latter case is 1.72.

Another important feature presented by the present series is the high value on the 17th hour of July 22. In the previous experiments which have dealt with foliar transpiring power, there has been a fall in the transpiring power value after the secondary rise. It is noticed that the evaporation during the afternoon is rather intense, being 1.6. The high value of the transpiring power, therefore, is without question due to the high evaporating power of the air.

In formulating a graph (fig. 3) from the data presented in the march of foliar transpiring power, the general feature is the high foliar transpiring power before the time of greatest diurnal evaporation. For both leaves the maximum is reached at the 11th hour, when the index is 1.00. This value is retained for Ib_1 until the 12th hour, and for Ib_2 until the 13th hour. The minimum value (0.47) for Ib_1 occurs on the 22d hour, while for Ib_2 (0.44) it occurs on the 18th hour. The ratio of maximum to minimum or of day value to night value is 2.1 for Ib_1 and 2.3 for Ib_2. The sudden drop in the afternoon reading on the 14th hour is equally as striking as that

TABLE IV

DATA FOR MARCH OF INDICES OF FOLIAR TRANSPIRING POWER FOR *Helianthus* PLANT 1*b* (MAXIMA IN BOLD FACED TYPE, MINIMA IN ITALICS)

LEAF NUMBER	TIME OF OBSERVATION	TIME OF COLOR CHANGE IN SECONDS		INDEX OF TRANSPIRING POWER			EVAPORATION FROM STANDARDIZED ATMOMETER, CC. PER HOUR
		Lower surface	Upper surface	Lower surface	Upper surface	Entire leaf	
	July 21						
$1b_1$	18:25	27	63	0.89	0.38	0.64	.....
$1b_2$	18:25	44	73	0.55	0.33	*0.44*	
$1b_1$	19:25	40	67	0.72	0.43	0.58	0.22
$1b_2$	19:30	44	81	0.65	0.35	0.50	
$1b_1$	20:25	41	68	0.71	0.43	0.57	0.15
$1b_2$	20:25	43	78	0.67	0.37	0.52	
$1b_1$	21:15	44	90	0.66	*0.32*	0.49	0.16
$1b_2$	21:20	39	105	0.74	*0.28*	0.51	
$1b_1$	22:20	52	95	*0.60*	0.33	*0.47*	*0.09*
$1b_2$	22:25	50	97	*0.62*	0.32	0.47	
$1b_1$	23:15	47	85	0.68	0.38	0.53	0.15
$1b_2$	23:20	43	82	0.74	0.39	0.57	
$1b_1$	24:20	53	78	0.62	0.42	0.52	0.18
$1b_2$	24:20	49	73	0.67	0.45	0.56	
	July 22						
$1b_1$	1:20	51	78	0.67	0.44	0.56	0.12
$1b_2$	1:20	47	71	0.72	0.48	0.60	
$1b_1$	2:20	57	75	0.63	0.48	0.56	0.15
$1b_2$	2:20	50	72	0.72	0.50	0.61	
$1b_1$	3:20	46	70	0.78	0.52	0.65	0.15
$1b_2$	3:20	41	70	0.88	0.52	0.70	
$1b_1$	4:20	49	90	0.73	0.40	0.57	0.15
$1b_2$	4:20	43	75	0.84	0.48	0.66	
$1b_1$	5:20	48	82	0.75	0.44	0.60	0.15
$1b_2$	5:25	40	85	0.90	0.38	0.64	
$1b_1$	6:20	42	50	0.81	0.68	0.75	0.15
$1b_2$	6:20	46	55	0.74	0.62	0.68	
$1b_1$	7:15	31	37	0.74	0.62	0.68	0.15
$1b_2$	7:15	26	42	0.89	0.55	0.72	
$1b_1$	8:25	25	31	0.92	0.74	0.84	0.15
$1b_2$	8:25	25	28	0.92	0.82	0.87	
$1b_1$	9:10	25	30	0.84	0.70	0.77	0.22
$1b_2$	9:15	21	22	1.00	0.95	0.98	
$1b_1$	10:10	25	26	0.84	0.81	0.83	0.58
$1b_2$	10:10	23	24	0.91	0.88	0.90	
$1b_1$	11:10	19	19	**1.00**	**1.00**	**1.00**	.....
$1b_2$	11:10	19	19	**1.00**	**1.00**	**1.00**	
$1b_1$	12:10	18	18	**1.00**	**1.00**	**1.00**	.....
$1b_2$	12:10	18	18	**1.00**	**1.00**	**1.00**	
$1b_1$	13:15	16	16	0.93	0.93	0.93	1.0
$1b_2$	13:20	15	15	**1.00**	**1.00**	**1.00**	
$1b_1$	14:15	14	15	0.71	0.67	0.69	1.2
$1b_2$	14:20	17	17	0.59	0.59	0.59	
$1b_1$	15:20	15	15	0.93	0.93	0.93	1.2
$1b_2$	15:20	16	17	0.88	0.82	0.85	
$1b_1$	16:25	16	18	0.94	0.83	0.89	**1.6**
$1b_2$	16:25	17	19	0.88	0.79	0.84	
$1b_1$	17:10	19	18	0.95	1.00	0.98	1.0
$1b_2$	17:15	20	23	0.90	0.78	0.84	
$1b_1$	18:10	27	30	0.74	0.67	0.71	1.0
$1b_2$	18:15	30	33	0.67	0.61	0.64	

noted for series I*a*. For Ib_1 the drop really begins on the 12th hour and falls from 1.00 to 0.69, giving a difference of 0.31; for Ib_2 the fall is from 1.00 to 0.59, giving a difference of 0.41. The recovery for Ib_1 is from 0.69 to 0.93, and for Ib_2 is from 0.59 to 0.85. The difference value is 0.24 in one case and 0.26 in the other. It was pointed out for series I*a* that the recovery of the older leaf is more marked than that of the younger leaf. This

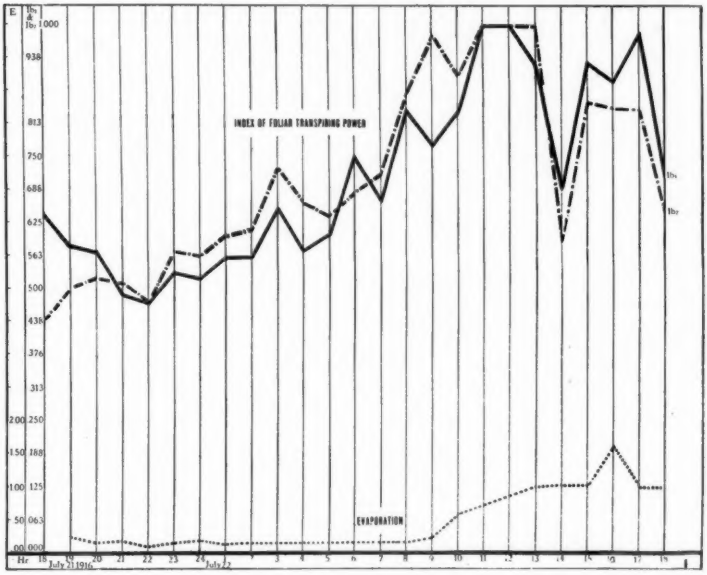


FIG. 3

feature is again borne out in the present series, where the values for Ib_1 are in excess of those of Ib_2.

For the reason that the leaves of series Ib_1 are very nearly of the same age, the same variation as set forth in the previous season will not be in evidence. The minimum values are slightly lower. As a result the ratios between maxima and minima are respectively 2.19 and 2.27. The same high foliar transpiring power is present at the 17th hour. This agrees with the former series. The data submitted in table V give the march of foliar transpiring power during the process of wilting from July 24–August 7.

TABLE V

INDICES OF FOLIAR TRANSPIRING POWER DURING PROGRESS OF WILTING OF *Helianthus* PLANT Ia

Time of observation			Index of transpiring power entire leaf		Evaporation from standardized atmometer, cc. per hour
			Ia_1	Ia_2	
July	24	9:15	0.51	0.68	1.00
		14:00	0.53	0.54	1.23
		20:00	0.28	0.39	0.73
July	25	9:10	0.49	0.29	0.24
		14:00	0.27	0.23	0.71
		21:00	0.59	0.31	0.66
July	26	9:25	0.51	0.31	0.11
		14:10	0.70	0.20	0.91
		21:15	0.49	0.21	0.62
July	27	9:25	0.43	0.20	0.02
		14:05	0.65	0.18	1.25
		22:25	0.69	0.18	0.51
July	28	9:00	0.38	0.16	0.12
		14:35	0.22	0.14	1.12
		21:00	0.36	0.19	0.66
July	29	9:20	0.33	0.18	0.13
		14:00	"	0.15	0.80
		21:00	"	0.13	0.69
July	30	9:05	"	0.21	0.28
		14:15	"	0.12	0.18
		21:00	"	0.16	0.91
July	31	10:05	"	0.34	0.26
		14:15	"	0.17	1.11
		21:05	"	0.09	0.66
August	1	10:05	"	0.17	0.24
		14:00	"	0.15	0.71
		21:05	"	0.13	0.41
August	2	10:10	"	0.14	0.24
		14:10	"	0.15	1.09
		22:45	"	0.16	0.53
August	3	10:00	"	0.14	0.34
		14:00	"	0.12	1.50
		23:00	"	0.16	0.37
August	4	10:00	"	0.17	0.22
		14:00	"	0.12	1.03
		21:00	"	0.17	1.18
August	5	10:10	"	0.17	0.33
		14:05	"	1.17	0.77
		21:00	"	0.11	0.21
August	6	9:30	"	0.14	0.11
		14:10	"	0.11	1.49
		21:00	"	0.13	1.10
August	7	10:00	"	0.13	0.49
		14:05	"	0.23	0.74

From the tabulated data of table V, and from graph (fig. 4) of series I*a*, it is noticed that there is a marked decrease in foliar transpiring power from July 24 up to the time when the plant wilts. The transpiring power of leaf Ia_1 is very irregular. There is no doubt but that the plant has attained its permanent wilting point on July 29, but because the leaves of this series are somewhat older than Ia_2, and as they are located nearer the absorptive center their action will be more or less modified by the presence of the younger leaves at the tip. For the leaf Ia_2 there is a marked decrease in the foliar transpiring power from July 24 to July 30, the foliar transpiring power being especially high on August 1. This feature is probably in response to the exceedingly high temperature at that time. The evaporation from the standardized atmometer bears out this statement. From August 1 to August 7 the index of foliar transpiring power proceeds almost in a straight line, except for small dips occurring in the majority of

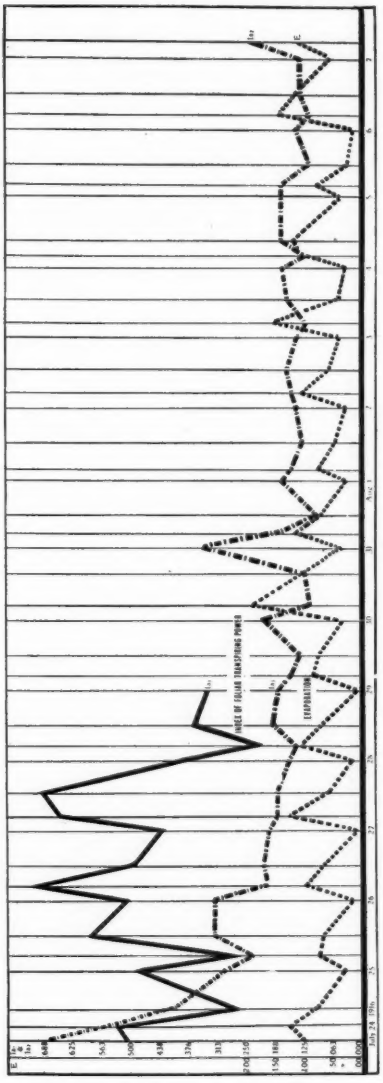


FIG. 4

cases when the evaporation was at its highest. This part of the graph conforms with the one obtained when the plants were lifted from the soil. On August 7 the index of foliar transpiring power increases from 0.13 to 0.23, or 77 per cent from the 10th hour to the 14th hour. On the previous day it dropped from 0.14 to 0.11, while on the preceding day the two indices were the same. At no other time during the march was there such a great percentage increase.

In obtaining the ratio between the day reading and the night reading for 24 consecutive hours, the day readings were usually made between the 9th and the 10th hours. For the night readings there was no need of selection as only one reading was taken. Beginning with July 24, and continuing until July 29 (time of wilting), the transpiring power indices representing the day readings for Ia_1 are 0.51, 0.49, 0.51, 0.43, 0.38, 0.33, while the corresponding night values are 0.28, 0.59, 0.49, 0.69, 0.36, 0.33. The respective ratio values are 1.82, 0.83, 1.04, 0.62, 1.05. On July 21–22 the ratio between the reading on the 9th hour and the reading on the 21st hour is 0.88:0.52, or 1.7. For the entire 24-hour period the maximum and minimum ratio is 1.9. The only normal ratio is the first. It is interesting to note that for leaf Ia_1 the minimum is normally 0.50. During the progress of wilting the maximum does not get below this point until July 27; after that it is below the usual minimum.

From July 24 to August 7 (time of wilting) the corresponding indices for Ia_2 are present; for the morning 0.68, 0.29, 0.31, 0.20, 0.16, 0.18, 0.21, 0.34, 0.17, 0.14, 0.14, 0.17, 0.17, 0.14, 0.13; for the night 0.39, 0.31, 0.21, 0.18, 0.19, 0.13, 0.16, 0.09, 0.13, 0.16, 0.16, 0.17, 0.11, 0.13. The ratio of the day (morning) readings to the night readings is respectively 1.74, 0.94, 1.11, 0.84, 1.38, 1.31, 3.78, 1.31, 0.88, 0.88, 1.00, 1.54, 1.08. For July 21–22 the ratio of maximum to minimum for Ia_2 is 1.72. For the corresponding hours the ratio is 0.95:0.66, or 1.44. On this basis, therefore, the readings of the first day are normal, in that the ratio is approximately the same as for the maximum to the minimum on July 21–22 (1.72). Also the maximum values during the march of wilting, with the exception of the first reading, are all

below the minimum set during the daily march of foliar transpiring power for July 21–22.

The data presented in table VI give results that harmonize with those of table V. As was stated in connection with the march of

TABLE VI

INDEX OF FOLIAR TRANSPIRING POWER DURING PROCESS OF WILTING OF *Helianthus* PLANT 1*b*.

Time of observation		Index of transpiring power entire leaf		Evaporation from standardized atmometer, cc. per hour
		$1b_1$	$1b_2$	
July 24	9:20	0.17	0.15	1.00
	14:00	0.18	0.21	1.23
	20:40	0.18	0.14	0.73
July 25	9:00	0.12	0.20	0.24
	14:00	0.17	0.14	0.71
	21:10	0.19	0.21	0.66
July 26	9:35	0.12	0.28	0.11
	14:15	0.18	0.12	0.91
	21:00	0.35	0.19	0.62
July 27	9:30	0.10	0.13	0.02
	14:15	0.19	0.18	1.25
	22:00	0.34	0.20	0.51
July 28	9:25	0.22	0.13	0.12
	14:45	0.19	0.14	1.12
	21:00	0.12	0.22	0.66
July 29	9:30	0.25	0.16	0.13
	14:00	0.24	0.10	0.80
	21:00	0.14	0.11	0.69
July 30	9:10	0.31	0.14	0.28
	14:20	0.14	0.15	1.88
	21:00	0.26	0.15	0.91
July 31	10:10	0.26	0.14	0.26
	14:20	0.17	0.17	1.11
	21:15	0.13	0.10	0.60
August 1	10:10	.....	0.21	0.24
	14:00	.....	0.21	0.71
	21:10	.....	0.14	0.41
August 2	10:15	.....	0.17	0.24
	14:15	.....	0.16	1.09
	22:50	.....	0.12	0.53
August 3	10:10	.....	0.15	0.34
	14:00	.....	0.20	1.50

foliar transpiring for 24 consecutive hourly periods, the leaves selected here were closer together, and considering the relation

which is present between the leaves it would be expected that the variation would not be as great.

The data given in table VI show slowly decreasing values; however, the decrease is not marked. The highest foliar transpiring power for Ib_1 is 0.35, while the lowest is 0.10. The highest point as here set forth occurs on the 21st hour on July 26, while on the following day the index at the same time is 0.34. After that there is a slight fall, although this is not true for all the readings, for on July 30 the index is 0.31. Even at the time of wilting, the index at the morning hour is 0.26. From July 29 to July 31 the maximum values are approximately the same. This is also true of the minimum values. The last reading for the Ib_1 series occurs on July 31 and gives an index of 0.13.

For leaf Ib_2 the values are strikingly similar to those of the leaf situated just below it upon the stem. The highest transpiring power index for the entire time is only 0.28, and occurs at 9:35, July 26; while the minimum value 0.10 occurs on the 14th hour of July 29 and on the 21st hour of July 31. The data of table VI, represented graphically in fig. 5, show a gradual dropping off of the day maximum values from July 26 to July 30. From July 30 to August 3, with the exception of August 1, the graph of wilting is practically a straight line. On August 3 there is a marked increase, considering that the entire period has given a low index throughout (from 0.15 to 0.20, or an increase of 33.3 per cent). Usually during the march of foliar transpiring power a drop is registered at the 14th or 15th hour. On July 27 there is an increase of the index from 0.13 to 0.18 (38.46 per cent) and on July 31 from 0.14 to 0.17 (21.43 per cent). On account of the comparatively small deviation between the maximum and minimum values throughout, the increase in the transpiring power of one-third on August 3 becomes more significant than the graph shows (fig. 5).

The ratio between the day indices and night indices is presented as before, the readings of the 9th and 21st hours being used. On July 24 the morning reading is 0.17; on the following days the average foliar indices of transpiring power for leaf Ib_1 are 0.12, 0.12, 0.10, 0.22, 0.25, 0.31, 0.26. The corresponding night values are 0.18, 0.19, 0.35, 0.34, 0.12, 0.14, 0.26, 0.13. The corresponding

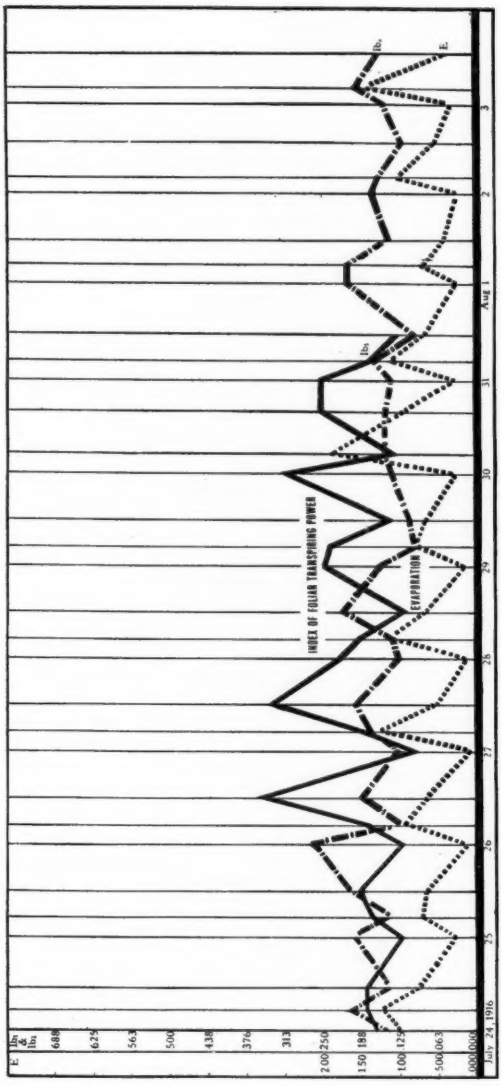

INDEX OF FOLIAR TRANSPIRING POWER
EVAPORATION
lbs.
E.
688
625
563
500
438
376
313
200 250
150 188
100 125
50 63
000 000
July 24, 1916
25
26
27
28
29
30
31
Aug 1
2
3

FIG. 5

ratios between the day and night readings are respectively 0.95, 0.63, 0.34, 1.83, 1.79, 1.19, 2.00. The ratio between the index of foliar transpiring power for the 9th and 21st hours on July 21–22 is 1.57. The ratio between the day maximum and the night minimum at that time is 2.11.

Taking into consideration that the minimum for Ib_1 during the march of foliar transpiring power on July 21–22 is 0.49, the maximum values during the march of wilting are extremely low from the initial to the final point of wilting. On account of the drop in the maximum and the constant retentive character of the minimum, the index here is larger than recorded previously. The leaf was completely wilted on July 31.

The day values for Ib_2 taken at the same time as before are 0.15, 0.20, 0.28, 0.13, 0.16, 0.14, 0.14, 0.21, 0.17, 0.15; while the corresponding night values are 0.14, 0.21, 0.19, 0.20, 0.22, 0.11, 0.15, 0.10, 0.14, 0.12. The ratios between these two sets are, in order of their occurrence, 1.07, 0.95, 1.45, 0.65, 0.59, 1.45, 0.93, 1.40, 1.50, 1.42. The ratio between the readings for the 9th hour and the readings of the 21st hour on July 21–22 for *Helianthus* leaf Ib_2 is 0.98:0.51, or 1.92. The ratio between the maximum and the minimum is 2.23. In none of these cases can the proportion be regarded as normal. This plant from the beginning is evidently in a partially wilted state.

In comparing the march of foliar transpiring power during the process of wilting for the two series I*a* and I*b*, there would naturally be some variation. The wilting of series I*a* extends over a longer period (to August 7), while that of I*b* reaches its permanent wilting point on August 3. In both cases the older leaf wilts long before the younger leaf. However, leaf I*a* has a greater range of foliar transpiring power. Leaf Ia_1 wilts before Ia_2; likewise Ib_1 before Ib_2.

The ratio between the morning and the night readings of each day gives in the majority of cases a value that is less than the normal. For I*a* all the results are either near 1.00 or below it except for the first. As the maximum value is above that of the usual minimum the result cannot be anything but normal. With I*a* the ratio on July 31 is extremely high, probably being due to the

extremely high evaporation. Why there should be such a decrease at the 21st hour is not known. The first ratio 1.74 is approximately equal to the normal. The day reading is 0.68. The ratio on August 5 is 1.54, but the 9th hour reading gives a value that is much lower than the usual minimum. With a slight decrease in the minimum, the ratio between the two becomes greater than before. From these data on the basis of the ratio between day and night foliar transpiring power values it is evident that, if the ratio is to be used during the process of wilting, it can only be applicable when the maximum is greater than the usual minimum. Throughout the series of both I*a* and I*b* the ratio does not deviate very far from unity, but in the formation of the ratio there is an evidently greater corresponding decrease in the day value as compared with the night reading. In both cases the extent of daily fluctuation for the younger leaves is very small after the first day.

The rate of evaporation throughout fluctuated considerably, but is unusually high for the climate of Chicago. There is nevertheless no close agreement between evaporation and foliar transpiring power during the march of wilting. Plants similar to the ones used in the experiment were treated like I*a* and I*b* and were placed in a moist chamber at their respective times of wilting. They behaved in a similar manner and failed to recover in the allotted time. Although the plants were watered at the same time with approximately the same amount of water, figs. 5 and 6 show indirectly that there was much difference in the soil moisture content. The plant designated as I*b* was larger than I*a*, and would be expected to wilt first. This observation is borne out in the experiment. It is also evident from an examination of the two graphs that the soil of I*b* was drier at the beginning than that of I*a*, as the indices of foliar transpiring power are much smaller.

STOMATAL DIFFUSION

The index of foliar transpiring power in its very definition is associated with that of vapor tension. The decrease in the index of foliar transpiring power such as is present at night during the daily march represents a great force. A solution may also carry with it just as great a force. LIVINGSTON (**27**), com-

menting upon FITTING'S (**19**) work upon the osmotic pressure present in desert plants, makes the statement that with the lowering of the vapor tension 10 per cent there is represented a pressure of 100 atmospheres. In an examination of the graph in table I there is a reduction in the index from 0.92 to 0.19 during the process of wilting. This therefore represents an approximate pressure of 800 atmospheres. For plant *B* there would be an approximate pressure of 700 atmospheres. Table V and fig. 4 give leaf Ia_1 as being able to withstand a pressure of 666 atmospheres and leaf Ia_2 860 atmospheres. Leaf Ib_1, with an index 0.10, suggests a pressure as high as 900 atmospheres. At that time the margin of the leaf was sufficiently dry so that the clip could not be used without causing injury. This status becomes all the more pertinent when it is compared with the data submitted by SHULL (**41**), where the force present in air dry seed (*Xanthium*) is equivalent to 1000 atmospheres.

During the daily march of foliar transpiring power there is usually considerable variation (figs. 2, 3), even when a plant is supplied with an optimum amount of water. The sudden rise in the foliar transpiring power immediately after sunrise, as set forth by BAKKE and LIVINGSTON, gives credence to the view that the stomata open quickly at this time. In using the porometer and standardized cobalt paper squares simultaneously, TRELEASE and LIVINGSTON (**42**) find that during the daily march there is considerable agreement between the porometer readings and the readings of the foliar transpiring power by the method of standardized cobalt chloride paper. From the results obtained in their investigation they concluded that the porometer gives readings which show the extent of stomatal diffusion.

DARWIN (**12, 13**), using the horn hygroscope and the temperature method, has shown that during wilting there is a temporary opening of the stomata. DARWIN and PERTZ (**14**), using the porometer, have demonstrated that a similar condition is present during wilting. LAIDLOW and KNIGHT (**26**) in their work upon stomatal behavior during wilting, where they employ a recording porometer, have confirmed the results of DARWIN and PERTZ, in that the stomata open temporarily during wilting. For *Phaseolus*

vulgaris the maximum diffusion occurred about 5 minutes after the leaf was severed from the stem, while in the case of the thick leaf of *Prunus Laurocerasus* nearly 20 minutes elapsed before the maximum stomatal opening was reached. KAMERLING (**23**) found that when *Rhipsalis cassytha* had lost 1 to 4 per cent of its normal water supply, the amount of transpiration per unit time increased, and later when the loss in weight had reached a certain point, varying from 6 to 10 per cent, the transpiration again diminished. KAMERLING is of the opinion that the increase in transpiration is due to the opening of the stomata. LLOYD (**31**), on the other hand, failed to find this temporary opening.

The evidences at hand support the conclusion that the stomata open for a short time during wilting. The time is short, however, and there is no evidence that the stomata ordinarily open up during the early stages of wilting and continue to be open until the plant has attained its permanent wilting point. This point is important in connection with argument presented for the break in the water columns.

In order to prove that the stomata open only during the early stages of wilting, the porometer as modified and used by KNIGHT (**24**) was resorted to. The plant was attached to the aspirator and allowed to remain until the leaves were partially wilted. Tests were made upon plants grown in the greenhouse and later transferred to the laboratory and plants which had been grown continuously in the greenhouse. The plants in the laboratory were kept for 5 days before experimentation was begun. The tests of this series were begun on November 28, 1917, and continued until December 3, 1917; readings were made at three different times of the day. This conforms with the plan adopted in making the readings of the foliar transpiring power. Evaporation was recorded by means of a standardized cylindrical form of atmometer of the Livingston type. The data are given in table VII.

In an examination of the data presented in table VII, readings were not taken until the 16th hour on November 29, 3 days after all watering had ceased. The time elapsing between two successive bubbles, as ascertained by means of a stop watch, was found to be 160 seconds. At the 11th hour it took 140 seconds, while

on December 1 at 10:30 it took 246 seconds. From that time until December 3 there was not much variation either at night or during the day.

TABLE VII

POROMOTER READINGS DURING PROGRESS OF WILTING OF A *Helianthus annuus* PLANT GROWING IN LABORATORY

Time of observation	Rate of evaporation from standardized atmometer, cc. per hour	Rate of flow, time interval in seconds between successive bubbles
November 27 16:00....	.....	.....
28 8:00....	.....	.....
28 15:30....	1.45	.....
29 16:00....	0.72	160
30 11:00....	1.12	140
30 14:30....	0.79	150
30 20:00....	1.34	146
December 1 10:30....	0.97	246
December 1 14:30....	1.11	266
December 1 20:00....	0.54	259
2 10:30....	1.79	276
2 14:30....	1.71	260
2 20:00....	1.41	262
3 10:30....	1.70	269

The data of the series grown continually in the greenhouse, where the evaporation was very low, are given in table VIII.

These results, which were obtained at regular intervals on 5 successive days (November 29 to December 3, 1917), do not show any marked differences. Considering the experimental error which would be present, there is not sufficient difference in any one case to indicate that stomatal movement was present. The plants used in this series were not watered for 3 days before the beginning of the experiment.

The stomatal diffusion as measured by the porometer was also determined for plants which were grown for the same period, but were not subjected to any extended period of wilting. The general average for the stomatal diffusion as represented by the time interval between successive bubbles of the air intake tube of a porometer was found to be 105 seconds. No attempt was

made to ascertain whether or not the stomata were partially closed or whether there was an increased opening after a period of 2 hours, as has been shown for certain plants by ILJIN (**20**). At any rate, the time interval in the case of intensely wilted *Helianthus* plants is much smaller. Even if the stomata should be partially

TABLE VIII

POROMETER READINGS TAKEN OF A WILTING *Helianthus* PLANT GROWING WHERE EVAPORATING POWER OF AIR IS LOW

Time of observation	Rate of evaporation from standardized atmometer, cc. per hour	Rate of flow, time interval in seconds between successive bubbles
December 11 16:00....	.....	.....
12 13:00....	0.35	.....
12 16:30....	0.23	.....
13 10:30....	0.34	.....
13 14:30....	0.65	.....
13 20:00....	0.45	.....
14 10:30....	0.37	357
14 14:30....	0.60	360
14 20:00....	0.34	352
15 10:30....	0.30	318
15 14:00....	0.36	309
15 20:00....	0.40	335
16 10:30....	0.43	357
16 14:30....	0.43	348
16 20:00....	0.49	343
17 10:30....	0.40	341
17 14:30....	0.44	365
17 20:00....	0.37	343
18 10:30....	0.34	398
18 14:30....	0.44	389
18 20:30....	0.39	360

closed, the results obtained from tables VII and VIII show that during the march of wilting, where the plant acquires its permanent wilting point, the stomatal opening does not enter in to affect the diffusion or transpirational water loss. This statement is in agreement with that of DARWIN, that when the transpiration is high and the supply water insufficient the lack of water is a more important factor than stomatal changes. It would be extremely advantageous, however, to have the stomatal movement question

settled. Regarding the stomatal diffusion as a minor factor during intense wilting, the problem resolves itself to the point where the resistance to the passage is considered. From the data given in this paper and in a previous publication the resistance is exceedingly great. This will give further information, therefore, upon the strength of the evaporating force and that of cohesion.

Discussion

In comparing the results obtained during the summer of 1915 with those of 1916, considerable additional evidence is set forth which substantiates the argument advanced by BAKKE that wilting occurs at a definite point and is readily determined by the use of standardized hygrometric paper. In the series of 1915 the average of 3 leaves were used in plant I*a* and 2 leaves for plant I*b*. No effort was made during the 1915 season to obtain the difference in the time of wilting for leaves of different ages. The difference, however, was probably very slight, as the evaporation was exceedingly low. At no time during the entire run was the evaporation as high as 0.7 cc. per hour, and usually it was below 0.5 cc. per hour. The temperature of the greenhouse was seldom over 28° C.

In contrast, the evaporation during the season of 1916 was high and during the time the experiments were being performed was exceedingly uniform. It may be added that during the progress of the experiment no rain fell. It would then have been preferable to have run the experiments outside, but in the climate of Chicago it is rather difficult to obtain such a continued period of clear weather. The usual feature will then be a low evaporation at night, a higher one during the forenoon, and the maximum at the 14th hour. The high evaporation rate on July 31 is not explainable. It may be well to remark, however, that on July 30 the temperature in the greenhouse was 41.2° C. and at the first hour of July 31 it was 27° C., almost the maximum of the previous year.

It is again brought out that for the 1915 and 1916 series a point is reached where the foliar transpiring power shows very little fluctuation. In the cases presented, this point can be represented graphically by a line that is almost straight. The ratio values are not far above unity in the majority of cases, and sometimes are

even lower. It is noted also that the time element of this period varies greatly in the two seasons. In 1915 it is comparatively short, while for both series in 1916 it is extended over a considerable period. It has been proved by the work of SHREVE (**34**) that plants grown under different environment not only have different anatomical characters but also have a different rate of transpiration.

On the basis therefore of a possible change as a result of environment, it can safely be asserted that this is the reason for the short span in 1915 and the long one in 1916. Why or how the plant establishes such an apparent equilibrium cannot be stated. This equilibrium represents the greatest force or tension which can be applied before a plant assumes the condition of permanent wilting. A plant such as *Atriplex* will necessarily have an extended period when this equilibrium is maintained. The exact wilting will be when there is a serious rupture in the water columns.

If this interpretation is correct, the 1915 and 1916 series should exhibit a difference in the foliar transpiring power values during the so-called equilibrium stage. It would be expected that the 1915 series would have a higher minimum than the 1916 series. This is evident, for in 1915 the lowest point reached at any time is never below 0.15, while for the 1916 series it is as low as 0.09 in one case and 0.10 in the other. There would thus seem to be a direct relation between the time of the equilibrium, the lowest point in the index of foliar transpiring power, and the evaporating power of the environment. The point at which wilting occurs is definitely marked out. This point appears graphically to better advantage for the plants of 1915 than for those of 1916; but the plants of 1915 were larger and were grown in smaller containers than those of the following year. For the series of 1915 the permanent wilting occurs on August 21, while for series I*a* (1916) the wilting occurs on August 7, and for series I*b* of the same year the wilting occurs on August 4.

In this study the same conception of wilting is advanced as before. The present study is really more or less of an elaboration of the former. It is assumed here that DIXON'S (**16, 17, 18**) con-

ception of continuous water columns is in force. When the force of evaporation becomes sufficient to cause a serious rupture of these water columns, then the plant wilts. Just to what extent a serious rupture can be regarded cannot be stated, but it must be greater than the force of cohesion which holds the water particles together.

The extent of this cohesion force has been sufficiently presented and advanced by DIXON (**16, 17, 18**), RENNER (**34, 35, 36**), URSPRUNG (**43, 44, 45, 46, 47**), and others (**21, 32**), and although the conclusions have been criticized by JOST (**22**), nevertheless they are substantiated. It is not the province of this article to enter into a critical discussion of these various papers. The approximate point of permanent wilting is readily ascertained from the beginning by taking a series of readings of the foliar transpiring power of the plant in question. Care should be taken to obtain in the series the maximum and minimum. Although there is not any hard and fast relation between the maximum and the minimum, when the moisture in a soil has been reduced to the point where the maximum is below the normal minimum, at a time of the normal maximum, then the water content of that soil has attained what the writer designates as the critical content. From this point it is simply a question of time when the columns break. This then becomes a relatively simple matter.

The readings giving the indices of foliar transpiring power taken at hourly intervals present a graph that is similar to graphs set forth previously. The maximum occurs at a time previous to the highest evaporation; the minimum generally occurs somewhere between the 18th hour and the 24th hour. There is a decided drop in the afternoon, which occurs at a time of day when evaporation is at its height or nearly so. There is a recovery that is also conspicuous. The cause for this resistance has been advanced by SHREVE (**40**) as being due to the imbibitional forces of the cell wall and of the colloids of the protoplasm. Although this feature has been noticed wherever the march of foliar transpiring power has been obtained, no one as yet has set forth any evidence as to the length of time necessary for recovery to take place. It is apparent that the recovery has been complete before the time of the

beginning of the next reading, which in this case is the next hour. A record of the foliar transpiring power at hourly intervals at Chicago gives results that are similar to those obtained for plants of the same species in southern Arizona.

The series of 1916 show conclusively that the older leaves are the first to wilt. In an examination of series I*a* the older set of leaves is almost completely dry at the time of the permanent wilting of the plant. On July 29 the edges of the leaf are dry, but at the same time there is a different form of response in the younger leaves, in that the apparent recovery occasioned at the time of permanent wilting does not present itself. The same situation is true for the series I*b*, where the older leaves wilt on July 31. That the older leaves are the first to wilt has previously been determined by a number of investigations (**15, 33**). BAKKE and LIVINGSTON have presented evidence that there is considerable variation in the index of foliar transpiring power of young and old leaves. The fact that the younger leaves wilt later than the older leaves is not necessarily connected with the environment. This is true whether the evaporation is low or whether it is high. The production of the absciss-layer may at least be indirectly formed as a result. PRINGSHEIN (**33**) previously has shown that young leaves retain their freshness for a longer time than older ones. This he ascribes to a greater osmotic pressure. During the march of wilting it is also noticed that the foliar transpiring power index of the older leaves is always higher, at least than that of the leaves of the tip. The older leaves then give a higher foliar transpiring power throughout.

There is also in evidence during the march of wilting not only a low index of foliar transpiring power, but also a gradual increase of the force in opposition to the passage of water. When for a short time there is an evident break or a serious rupture, there is a decrease in the resistance, but an equilibrium with the atmosphere is soon reached. The assumption that there is a temporary opening of the stomata may be made at this point. Employing the porometer upon *Helianthus* plants placed in an environment of high evaporating power and one of low evaporating power, the author failed to find that the stomata are concerned.

Summary

1. The transpiring power of plants as determined by standardized hygrometric paper gives an accurate knowledge of the internal water relations of a plant. The exact wilting point as determined by this method occurs when there is a serious rupture in the water columns.

2. During the daily march of foliar transpiring power obtained by making consecutive hourly readings for 24 hours, the maximum is attained at a time previous to the greatest evaporation. During the time of approximate maximum evaporation there is a marked fall in the foliar transpiring power index, followed shortly by a rise. The ratio between the maximum and the minimum is more or less definite, but not sufficiently so for the formation of any law. When the ratio is reduced to the point where it is in the neighborhood of unity, the plant is in a state of intense incipient drying. When the maximum value does not exceed the usual minimum, the plant is in a soil environment which is critical from the point of water supply, or almost at its wilting coefficient. It is then merely a question of time before the plant wilts.

3. Evaporation plays an important part in the experiment upon transpiration. A high evaporation gives an increased transpiring power value, but during the process of wilting the index of foliar transpiring power comes to be independent of evaporation.

4. During the process of the march of wilting an equilibrium point is reached where the indices of foliar transpiring power do not show much variation. It is suggested that the duration of the equilibrium gives a measure of the comparative drought resistance of different plants. *Helianthus* grown in 1915 during a rainy season is different from *Helianthus* grown during 1916, when the season was unusually dry. The equilibrium period of 1915 was much shorter than for 1916.

5. There is a decided difference in the time at which permanent wilting occurs in old and young leaves. The older leaves will wilt long before the younger ones. The time interval varies according to age.

6. Stomatal movements or changes are not important factors when the plant is in an intense state of wilting.

I wish to express my thanks to Dr. H. C. COWLES and DR. G. D. FULLER, University of Chicago, who have aided me with suggestions and criticisms.

IOWA STATE COLLEGE
AMES, IOWA

LITERATURE CITED

1. ALWAY, A. J., Studies on the relation of the non-available water of the soil to the hygroscopic coefficient. Neb. Agric. Exp. Sta. Research Bull. 3. 1913.
2. BAKKE, A. L., Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. Jour. Ecol. 2:145–173. 1914.
3. ———, The index of foliar transpiring power as an indicator of permanent wilting in plants. BOT. GAZ. 60:314–319. 1915.
4. BAKKE, A. L., and LIVINGSTON, B. E., Further studies on the foliar transpiring power in plants. Physiol. Researches 2:51–71. 1916.
5. BRIGGS, L. J., and SHANTZ, H. L., Application of wilting coefficient determinations in agronomic investigations. Proc. Amer. Soc. Agron. 3:250–256. 1912.
6. ———, The wilting coefficient and its indirect determination. BOT. GAZ. 53:20–37. 1912.
7. ———, The relative wilting coefficients for different plants. BOT. GAZ. 53:229–235. 1912.
8. ———, The wilting coefficient for different plants and its indirect determinations. U.S. Dept. Agric., Bur. Plant Ind. Bull. 230. 1912.
9. ———, Die relativen Welkungskoeffizienten verschiedener Pflanzen. Flora 105:224–240. 1912.
10. BURGERSTEIN, A., Die transpiration der Pflanzen. Jena. 1914.
11. CALDWELL, J. S., The relation of environmental conditions to the phenomenon of permanent wilting in plants. Physiol. Researches 1:1–56. 1913.
12. DARWIN, F., Observations on stomata. Phil. Trans. Roy. Soc. Lond. 190:531–621. 1898.
13. ———, On a self-recording method applied to the movements of stomata. BOT. GAZ. 37:81–103. 1904.
14. DARWIN, F., and PERTZ, D. F. M., New method of estimating the aperture of stomata. Proc. Roy. Soc. Lond. B. 84:136–154. 1911.
15. DELF, E. MARION, Transpiration in succulent plants. Ann. Botany 26:409–442. 1912.
16. DIXON, H. H., Transpiration and the rise of sap. Prog. Rei Bot. 3:1–66. 1909.

17. DIXON, H. H., Transpiration and the ascent of sap in plants. London. 1914.
18. ———, Theoretisches und Experimentelles zur Kohäsiontheorie der Wasserbewegung. Jahrb. Wiss. Bot. **56**:617–667. 1915.
19. FITTING, HANS, Die Wasserversorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen. Zeitschr. Bot. **3**:209–75. 1911.
20. ILJIN, W. S., Die Probleme des vergleichender Studiums der Pflanzentranspiration. Beih. Bot. Centralbl. **32**:15–35. 1914.
21. HOLLE, HANS, Untersuchungen über Welken, Vertrocknen und Wiederstraffwerden. Flora **8**:73–126. 1915.
22. JOST, L., Versuche über die Wasserleitung in der Pflanze. Zeitschrift für Botanik **8**:1–55. 1916.
23. KAMERLING, Z., De Reguliering van de verdamping bij *Viscum album* in bij *Rhipsalis cassytha*. Ver. K. Ak. Ned. Amsterdam **22**:821–835. 1914.
24. KNIGHT, R. C., A convenient modification of the porometer. New Phytol. **14**:212–216. 1915.
25. ———, On the use of the porometer in stomatal investigation. Ann. Botany **30**:57–76. 1916.
26. LAIDLOW, C. G. P., and KNIGHT, R. C., A description of a recording porometer and a note on stomatal behavior during wilting. Ann. Botany **30**:47–56. 1916.
27. LIVINGSTON, B. E., The relation of the osmotic pressure of the cell sap in plants to arid habitats. Plant World **14**:153–164. 1911.
28. ———, The resistance offered by leaves to transpirational water loss. Plant World **15**:1–35. 1913.
29. LIVINGSTON, B. E., and BROWN, W. H., Relation of the daily march of transpiration to variation in the water content of leaves. BOT. GAZ. **53**:309–330. 1912.
30. LIVINGSTON, B. E., and SHREVE, EDITH B., Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant World **19**:287–309. 1916.
31. LLOYD, F. E., The physiology of stomata. Carnegie Inst. Wash. Publ. **82**. Washington. 1908.
32. NORDHAUSEN, M., Über die Saugkraft transpirierender Sprosse. Ber. Deutsch. Bot. Gesell. **34**:619–639. 1916.
33. PRINGSHEIN, E., Wasserbewegung und Turgorregulation in welkenden Pflanzen. Jahrb. Wiss. Bot. **43**:89–144. 1906.
34. RENNER, O., Zur Physik der Transpiration. Ber. Deutsch. Bot. Gesell. **29**:125–132. 1911.
35. ———, Experimentelle Beiträge sur Kenntnis der Wasserbewegung. Flora **103**:171–247. 1911.
36. ———, Versuche zur Mechanik der Wasserversorgung. Der. Druck in den Leitungsbahnen von Freilandpflanzen (Vorlaufige Mitteilung). Ber. Deutsch. Bot. Gesell. **30**:576–641. 1912.

37. SHIVE, J. W., and LIVINGSTON, B. E., The relation of atmospheric evaporating power to soil moisture content at permanent wilting in plants. Plant World **17**:81–121. 1914.

38. SHIVE, J. W., and JARTIN, WM. H., The effect of surface films of Bordeaux mixture on the foliar transpiring power in plants. Plant World **20**:67–86. 1917.

39. SHREVE, EDITH B., The daily march of transpiration in a desert perennial. Carnegie Inst. Wash. Pub. **149**. Washington. 1914.

40. ———, An analysis of the cause of variation in the transpiring power of cacti. Physiol. Researches **2**:73–127. 1916.

41. SHULL, C. A., Measurement of the surface forces in soils. BOT. GAZ. **62**:1–31. 1916.

42. TRELEASE, SAM F., and LIVINGSTON, B. E., The daily march of transpiring power as indicated by the porometer and by standardized hygrometric paper. Jour. Ecol. **4**:1–14. 1916.

43. URSPRUNG, A., Zur demonstration der Flüssigkeitskohäsion. Ber. Deutsch. Bot. Gesell. **31**:388–400. 1913.

44. ———, Über die Bedeutung der Kohäsion für das saffsteigen. Ber. Deutsch. Bot. Gesell. **31**:401–412. 1913.

45. ———, Filtration und Hebungskraft. Ber. Deutsch. Bot. Gesell. **33**: 112–117. 1915.

46. ———, Zweiter Beitrage zur Demonstration der Flüssigkeitskohäsion. Ber. Deutsch. Bot. Gesell. **33**:253–265. 1915.

47. ———, Über die Kohäsion der Wassers in Farnannulus. Ber. Deutsch. Bot. Gesell. **33**:153–162. 1915.

NOTES ON AMERICAN WILLOWS

I. THE SPECIES RELATED TO SALIX ARCTICA PALL.

CAMILLO SCHNEIDER

The more I advance in the study of American willows the more I realize that every species and form needs thorough investigation, and that even the most common and apparently best known species are far from being well understood in their variation and relationship to other forms. It will take two years more before I shall be sufficiently acquainted with all the American species hitherto described and preserved in the leading herbaria of this country to undertake their final arrangement in a monograph. At the advice of Professor SARGENT, therefore, I shall prepare, in the course of my studies, a series[1] of papers dealing with those species and forms which I have had an opportunity to investigate as thoroughly as can be done with herbarium material only. In November 1917 I commenced an investigation of the willows treated by RYDBERG in his paper entitled "Caespitose willows of Arctic America and the Rocky Mountains" (Bull. N.Y. Bot. Gard. 1:257. 1899). I received from the New York Botanical Garden and from the Herbarium of the Geological Survey of Canada at Ottawa the material that RYDBERG had before him. Besides this I had at my disposal the splendid collections of the Gray Herbarium, the Missouri Botanical Garden, and of course of the Arnold Arboretum. Furthermore, I was able to see the Labrador material of the Bebb Herbarium, now in the Herbarium of the Field Museum at Chicago, and also very interesting collections made in Labrador, Greenland, and Alaska from the Herbarium of Cornell University. I take this opportunity to offer my best thanks to the gentlemen in charge of all these herbaria. Unfortunately I have not been able to look over the rich collections of the U.S. National Herbarium at Washington.

It would have been of the greatest advantage if I could have seen the material collected by LUNDSTRÖM and used in his "Kritische

[1] For my first paper see BOT. GAZ. 65:1–41. 1918.

Bemerkungen über die Weiden Nowaja Semljas und ihren genetischen Zusammenhang" (Act. Reg. Soc. Sci. Upsala III. 1877), for without comparing a good series of specimens of *S. arctica* and *S. glauca* from Northern Asia and Europe it is difficult to get a correct understanding of those forms from North America; but at present it is impossible for me to consult any European herbarium.

In this article I shall try to present a critical account of the species related to *S. arctica* Pall.; in a following paper I intend to discuss *S. glauca* L. and the species related to it; while in a third paper a key will be given containing the species treated in the first two papers and also those of sections RETICULATAE and HERBACEAE (RETUSAE), together with a few other species the systematic position of which is not yet fully understood, but which are best placed near one or the other of the groups in question. In this key it is intended to indicate briefly the main characters of the species, because full descriptions cannot be given here except of the new species and varieties I wish to propose.

The history of most of the species must be explained, I am sorry to say, at considerable length, since otherwise it would be impossible to account for the fact that so many well marked types have been interpreted so differently by various authors. I commence with *S. arctica* Pall., which is the nucleus of the group of forms I shall try to elucidate.

1. S. ARCTICA Pall., Fl. Ross. 1^2:86. 1788.—PALLAS described this species from the "plaga arctica muscosa nuda secundum Sinum Obensem et versus glacialem Oceanum" in such an unmistakable manner that it could never have been misunderstood had not ROBERT BROWN, in 1819, proposed a new *S. arctica*, ignoring altogether the older name of PALLAS. In ROSS, Voy. Expl. Baffin's Bay (appendix, p. 148, and ed. 2, **2**:194, both in 1819), BROWN mentioned only the name, and a description of his *arctica* was first given by RICHARDSON in FRANKLIN, Narr. Jour. Polar Sea, Bot. App. 752 (reprint, p. 24). 1823. In the same year BROWN published his own description in Chloris Melvilliana, which was issued separately, while Capt. PARRY'S Voyage, of which the Chloris is only a part (App. Suppl. pp. 259–305), did not appear until 1824; but in 1823 there also appeared a second edition of FRANKLIN'S book and

RICHARDSON'S Appendix. Later, in the synonymy of *S. anglorum* Cham., I shall give the full and exact quotations of *S. arctica* Br.

The earlier *S. arctica* Pall. has also been overlooked by KOCH (1828), who mentioned only BROWN'S species. The first author who recognized the two discrepant *arctica* seems to have been MEYER (De Plant. Labrad. 32. 1830), where in a note to *S. arctica* Br. he says, "Quid est *Salix arctica* Pallas (florae rossicae II. pag. 170 editionis minoris)? Nullibi eam vel ut peculiarem speciem, vel ut synonymon apud botanicos memoratam inveni." In 1831 CHAMISSO (Linnaea **6**:541) proposed the name *S. anglorum* for *S. arctica* Br., non Pallas; see under *S. anglorum*.

In 1832 TRAUTVETTER, in his valuable study "De Salicibus frigidis Kochii," described the 3 following species: *S. crassijulis* Trev., *S. diplodictya* Trvt., and *S. torulosa* Led. Of these in 1833 (in LEDEBOUR, Fl. Alt. **4**:283) he referred *S. crassijulis* and *S. torulosa* as synonyms of *S. arctica* Pall., which had not been mentioned by him in 1832. In this year he described and figured only a *S. arctica* Br., which in 1833, however, he says is nothing but a synonym of *S. glauca* L. In MIDDENDORFF, Reise Sib. 1^2:27 (Florul. Taimyr.), TRAUTVETTER again changed his opinion, saying, "*Sal. arcticam* Pall. et *Sal. arcticam* R. Br. unam eandemque speciem sistere opinor. Planta, quam in dissertatione de Salicibus frigidis N. 7. tab. VI. sub nomine *Sal. arcticae* R. Br. proposui, ad *Sal. glaucam* L. referenda est nec sistit veram *Sal. arcticam* R. Br., uti e descriptione cel. R. Brownii in Fl. Melv. l.c. elucet." See also under *S. anglorum*.

In 1849–51 LEDEBOUR (Fl. Ross. **3**:619) included under *S. arctica* Pall. BROWN'S species as well as TRAUTVETTER'S 3 species of 1832, and also added to *S. arctica* such forms as var. *minor* (*S. phlebophylla* And.) and var. *leiocarpa* (*S. rotundifolia* Trev.). LEDEBOUR seems to have been the first author who mentions *S. anglorum* Cham. in the synonymy.

In a strange way the forms related to *S. arctica* have been treated by ANDERSSON (DC. Prodr. **16**^{2}:285. 1868), who, in 1858, in his previous work on North American willows, only mentioned *S. arctica* Br. as a "species difficile sane definienda, quasi inter *S. myrsinitidem* et *glaucam* prorsus media et formas plures ambiguas

amplectens." To those "formas ambiguas" belong the 3 varieties (*subphylicifolia*, *subreticulata*, and *subpolaris*) proposed by ANDERSSON in 1858, which I have not yet been able to interpret correctly owing to lack of the type material.

In the Prodromus, ANDERSSON created a *S. Pallasii* with the var. *crassijulis* (Trev.) and var. *diplodictya* (Trvt.), and mentioned, strange to say, the type of PALLAS under the last variety, while he is using the name *S. arctica* Pall. to cover a multitude of forms including his var. *nervosa*, *Brownei*, *groenlandica*, *petraea*, and *taimyrensis*. He excluded from his *S. arctica*, therefore, the forms of the true *S. arctica* Pall., and combined under this name a series of very different things like *S. altaica* Ldstr. (recte *S. torulosa* Led.), *S. anglorum* Cham., *S. groenlandica* Ldstr., *S. petrophila* Rydbg., *S. taimyrensis* Trvt., and others.

The first who attempted to clear up the *Pallasii-arctica* mixture of ANDERSSON was LUNDSTRÖM in 1877, in his interesting study previously mentioned. He confined *S. arctica* Pall. to its typical forms, and distinguished besides *S. Brownei* (And.) Ldstr., for which *S. anglorum* Cham. is the oldest name, *S. groenlandica* (And.) Ldstr., and *S. altaica* Ldstr., in which case he overlooked the priority of *S. torulosa* Led., a species founded on the same type. LUNDSTRÖM did not use CHAMISSO'S name because, following ANDERSSON, he referred *S. anglorum* to *S. phlebophylla;* see under *S. anglorum*. Another attempt to interpret properly *S. arctica* Pall. and *S. arctica* R. Br. was made by BEBB (BOT. GAZ. **14**:115. 1889), who, however, did not know LUNDSTRÖM'S work. Consequently he proposed another *S. Brownii* which, sensu stricto, corresponds with *S. anglorum*, a name likewise overlooked by BEBB, who refers some different forms to his *Brownii*. In 1899 BALL (Trans. Acad. Sci. St. Louis **9**:89) mentioned that "the methods by which Professor ANDERSSON succeeded in greatly augmenting the then existing confusion in regard to *S. arctica* R. Br. and *S. arctica* Pall. have been exposed by Mr. BEBB," and stated that BEBB had ignored the existence of LUNDSTRÖM'S earlier homonym; but BALL, in his turn, overlooked the name given by CHAMISSO many years before. It was RYDBERG who, in 1899, reinstated the name *S. anglorum* as the oldest correct name for *S. arctica* Br., non Pall.

I have seen but one leaf of the type specimen of PALLAS. It does not possess stomata in the epidermis of the upper surface, a character upon which I am inclined to lay considerable stress. It is true that by A. and E.-C. CAMUS (Class. Saules Europe **2**:55. 1905) *S. arctica* is said to possess "stomates ... assez nombreux" in the upper leaf epidermis, but judging by their synonymy these authors include under *S. arctica* so many widely different forms that they probably did not examine a true *arctica* at all. So far as I can see, this species is represented in the New World only in Alaska, the Yukon Territory, and the adjacent part of the northwest corner of British Columbia, and in the apparently well marked var. *subcordata* in southern British Columbia. I am not yet quite sure how far the range of *S. arctica* extends toward the east, but it seems not to cross 130° W. longitude except in the var. *subcordata*, of which the geographical distribution is not yet fully known. The specimen collected by BELL on Nottingham Island, Hudson Strait (no. 24623 O.[2] olim 18825), which is cited by RYDBERG under *S. arctica* Pall., belongs certainly to *S. anglorum*.

There seems to be no great difficulty in distinguishing typical forms of *S. arctica* from those of *S. anglorum* if one has well developed specimens. Very often, however, it is necessary to deal with mere fragments, and in this case the best character seems to be furnished by the presence or absence of stomata in the upper leaf surface. While they are entirely lacking in what I take for typical *S. arctica*, they are more or less numerous in all the specimens I have seen of *S. anglorum*. Generally, *S. arctica* is a much more robust plant with larger leaves and catkins and thicker branchlets, but when we compare the shape and pubescence of the leaves and the different characters of the flowers and fruits it is rather difficult to express in words those signs that the eye can more or less easily perceive. The best description of the American form of *S. arctica* is given by COVILLE in his excellent study of the "Willows of Alaska" (1901), to which is added a good plate. I shall say something more about the differences between *S. arctica* and *S. anglorum*

[2] In citing herbarium specimens I use the same abbreviations as in my first paper; see BOT. GAZ. **65**:9. 1918. There are to be added the following: C., Herb. Field Columbian Museum; Cor., Herb. Cornell University; O., Herb. Geol. Surv. Canada.

under the latter species; otherwise I refer to the keys that will be given in my third paper.

Regarding the variability of *S. arctica*, COVILLE said: "The large number of specimens examined tends to confirm the idea that the extreme variation in the leaves is chiefly an individual characteristic and does not mark recognizable incipient species. The nearest approach I have found to a subspecific differentiation is in some of the specimens from the Pribilof and St. Matthew Islands in Bering Sea, and the Shumagin Islands. In these specimens the leaves are orbicular, or nearly so, and only about 2–3 cm. in diameter, while the catkins are shorter than usual, about 1.5–3.5 cm. in length." These forms represent ANDERSSON'S *S. Pallasii* α, *crassijulis* 3 *obcordata* (1868) (*S. Pallasii* var. *obcordata* Turner; *S. arctica obcordata* Rydb.), who also distinguished f. *grandifolia* and f. *oblongata* of his var. *crassijulis*. The last two forms are, I believe, without any taxonomic value, while f. *obcordata* well deserves to be mentioned as a form or even as a variety. It differs chiefly in the characters mentioned by COVILLE. In addition to the localities cited by this author, I saw specimens from the Yacutat Bay, Glacier Bay (Muir Glacier), and Unalaska which should be referred to var. *obcordata* (And.) Rydbg. The following extract from an account given by TURNER (Contrib. Nat. Hist. Alaska 75. 1886) seems to me worth quoting.

> *S. Pallasii* Anders. var. *obcordata* Anders. This species of willow attains the largest size of any among the Aleutian Islands. The growth is exceedingly crooked, rarely straight for more than a foot, attaining a diameter of 2 to 3 inches, but often decayed within. In all the valleys and wider ravines this species is found in abundance. The roots form an intricate mass, often much exposed, and with the crooked branches and trunks form an impenetrable thicket of considerable area. . . . . VEMAMINOF [a Russian traveler] states that in former years this willow grew to such a size in one of the ravines opening on the west side of Captain's Harbor at Unalaska Island that the Russians and Aleuts procured sufficient of these trunks to be used advantageously in making bidaras (open skin boats). . . . . I visited the locality to find traces of such former growth and found the willows to be of but little better size than in other places near by.

There is another form which has very glabrescent capsules and may be identical with *S. arctica* var. *glabrata* Trautvetter (Act. Hort.

Petrop. **5**:107. 1877), of which he remarks: "Solum modo ovariis et bracteis parce puberulis a var. *typica* recedit. Forsan *S. arcticae* proles hybrida." Without having seen TRAUTVETTER'S type, which had been collected by CZEKANOWSKI and MUELLER "inter fl. Olenek et fl. Lena inferiorem, ad fl. Tyria in tundra," I am not sure whether the following plants really represent TRAUTVETTER'S variety: Unalaska, Kiuliuk, September 30, 1871, *M. W. Harrington* (fr.; G.), Dutch Harbor, July 17, 1899, *B. E. Fernow* (f., fr.; Cor.), Kodiak Island, July 2–4, 1899, *B. E. Fernow* (f., m.; Cor.), and Yakutat Bay, Disenchantment Bay, August 13, 1892, *F. Funston* (no. 117 partim, fr.; Cor., M., N.). LEDEBOUR (Fl. Ross. **3**:619. 1849–51) has described a variety with entirely glabrous fruits under the name var. *lejocarpa*, the type having been collected by ERMAN in Kamchatka in "ignivomo Schiwelutsch" (Shivelutch). This specimen, which I have not yet been able to compare, was mentioned by CHAMISSO (Linnaea **6**:541. 1840) as a form of *S. arctica* Pall., while ANDERSSON (1868) referred it to his *S. Pallasii* var. *diplodictya*. The true *S. diplodictya* Trautv. came from the "insula St. Laurent," and its main difference from typical *S. arctica* is, according to the author's description and figure, the "folia . . . . subtus pallidiora nec glauca nec glaucescentia, utrinque lucida." I have seen no specimen with such leaves, but COVILLE states that "occasionally specimens are found which lack the glaucousness of the lower leaf surface, a character on which TRAUTVETTER based chiefly his separation of *diplodictya*." RYDBERG, who kept *diplodictya* as a species, interpreted it in a very different way, and referred to it certain forms of which I shall speak under *S. ovalifolia*.

The var. *subcordata* previously mentioned from southern British Columbia is a form that needs further investigation. It has been described by ANDERSSON in Öfvers. K. Vet.-Acad. Förh. **15**:128 (Bidr. Känned. Nordam. Pilart.). 1858; in Proc. Amer. Acad. **4**:69 (Sal. Bor.-Am. 24). 1858; in Walp., Ann. Bot. **5**:754. 1858, from specimens collected by DRUMMOND in the "Rocky Mountains." In 1890 BEBB (BOT. GAZ. **15**:55) dealt with this rather obscure plant and stated that "the specimens from which the description of this supposed new species was drawn are all attached to a single sheet

in the Kew herbarium; they belong to three distinct and well known species." BEBB had received, through BAKER, nothing but a drawing and copies of the labels and "a few fragments, a capsule or two, to show minute characters." Through the kindness of Sir DAVID PRAIN the Arnold Arboretum has received (together with a series of photographs of other *Salix* types of the Hookerian herbarium) an excellent photograph of the type sheet of *S. subcordata* and also fragments of leaves and flowers. According to this material the fact stands as follows. In the upper left corner there are "two large specimens of *S. arctica* Pall." which BEBB believed had been labeled "almost certainly by some mistake" as "from the Rocky Mts. coll. *Drummond*" because, as BEBB had explained before, "nothing approximating in character to *S. subcordata* And. has been found." There are before me, however, the excellent specimens mentioned later from the Chilliwack Valley, which, in my opinion, are identical with DRUMMOND'S plant. Where this collector obtained his material I cannot ascertain, and so far as I know, he did not collect in this part of British Columbia. Beneath these two "*arctica*" specimens there are 3 (not 2, as BEBB stated) pieces, of which the one in the left corner is sterile, while the middle one bears female and the right one male flowers. Of both of them the Arboretum received fragments which show that they represent the same species as the older branchlets above them. BEBB refers those flowering branchlets to "*S. cordifolia* Hook.," and he is right in so far as HOOKER included in his *cordifolia* those Rocky Mountain forms. But ANDERSSON separated in 1858 just those western forms as *S. subcordata* from the eastern ones, which he then named *S. alpestris americana* (*S. cordifolia* Hook., pro parte).

The most critical part of the type sheet is the two sterile right hand branchlets which BEBB stated to be "two stunted specimens of *S. adenophylla*, leaves only, habitat not given." To those branchlets refers Dr. BARRATT'S label: "no. 92, *S. cordifolia* β *serrulata*." Of those pieces the Arnold Arboretum did not receive fragments, but, so far as I can judge by the photograph and by the corresponding number in Herb. N., they do not at all belong to *S. adenophylla* sensu BEBB (*S. syrticola* Fern.), but seem to represent a form of *S. Barclayi*, which grows together with *arctica subcordata*,

at least in the Chilliwack Valley. This fact explains the confusion of the two species, and there is to me no great "mystery how they came to be placed together on the same Kew sheet." It is, however, "more inexplicable how so critical a salicologist as ANDERSSON should have been misled into combining the characters of the two in his *S. subcordata*." I have also seen the "corresponding numbers of the HOOKER, BARRATT, and TORREY distribution in the Torrey Herbarium" mentioned by BEBB. There are 3 sheets before me. One contains a large leaf and 2 sterile young branchlets, and all 3 pieces belong to var. *subcordata*. This sheet bears the following 2 labels: "No. 90. Herb. H.B. [&T., crossed out; instead of it is written beneath "fig."] *S. obovata* var. *glabra*," and "88 Barratt, Rocky Mts. Ament leafy at the base about 4 leaves—Smooth and paler beneath." Underneath the big leaf BEBB, in 1887, has written "*S. crassijulis* Trev.?, *S. subcordata* And. in part." The second sheet contains two flowering branchlets and bears the label "No. 89 Herb. H.B. & T. Rocky Mts." as well as the statement in BEBB's handwriting "*S. subcordata* And. in part." These flowering branchlets are identical with those in Herb. Kew. The third sheet bears the *Barclayi* form previously mentioned.

ANDERSSON apparently had no clear idea of his *S. subcordata;* in 1858 he stated, "Quoad habitum quasi hybrida a *S. cordata* (cujus folia habet sed breviora) et *S. glauca* (amenta!)." In 1868 he referred to it some more specimens collected by BOURGEAU and DE LA PYLAIE, which I have not yet seen. The material before me looks very much like other robust specimens of *S. arctica*, but the leaves possess some stomata in the upper surface, at least along the main nerves. I think, therefore, that it is best to keep these forms as a variety of *S. arctica*, and I use ANDERSSON's name.[3]

S. ARCTICA var. **subcordata** (And.) nov. var. seems to differ from typical *arctica* chiefly by the following characters: foliis maximis obovato-ellipticis ad 8:6 cm. vel obovali-oblongis ad 7:2.5 cm. vel ellipticis ovali-ellipticisve ad 8.5:5 cm. magnis

[3] RYDBERG (Fl. Rocky Mts. 167. 1917) uses the name in a different sense. I am not yet quite sure what form is meant by him.

superne stomatiferis; amentis (saltem fructiferis) permagna ad 11 cm. longis et 1.5 cm. crassis.

As already stated, the exact locality where DRUMMOND collected the type is unknown to me. The other material came from British Columbia: Chilliwack Valley, between latitude 49°–49° 10′ and longitude 121° 25′–122°, 1650 m., August 29, 1901, *J. M. Macoun* (no. 26909 O., fr.; G., N.); Selese Mt., 1290 m., July 25, 1906, *W. Spreadborough* (no. 79556 O., fr.; Cor., N.; 79557 O., m.; Cor., N.; 79558 O., f., fr.; Cor.; 79559 O., m., fr.; Cor.); Skeena River, Hazelton Mountains, July 13, 1917, *J. M. Macoun* (no. 95405 O., f.).

There is also a forma incerta foliis oblongo-ellipticis utrinque acuminatis, collected by *G. E. Cooley*, in Juneau, Alaska, above Silver Bow Basin, August 6, 1891 (m.; G., N.), which has been referred by RYDBERG to *S. anglorum*. In my opinion it has nothing to do with that species, but may represent a special form of *S. arctica*, under which species it is cited by COVILLE.

2. S. ANGLORUM Chamisso in Linnaea **6**:541. 1831, exclud. specim. citat.—*S. arctica* R. Brown in Ross, Voy. Expl. Baffin's Bay, app. p. 143. 1819, and ed. 2. **2**:194. 1819, nomen nudum, non Pallas; Chloris Melv. 24. 1823; Capt. Parry's Voy. App. Suppl. p. 282. 1824; Richardson in Franklin, Narr. Jour. Polar Sea 752 (reprint 24). 1823; ed. 2. 765 (reprint 37). 1823.—*S. arctica* β *Brownei* Andersson in DC. Prodr. **16**²:286. 1868, pro parte.—*S. Brownei* Lundström in Nova Act. Reg. Soc. Sci. Upsala III. 1877. 37, pro parte max.—*S. Brownii* Bebb in BOT. GAZ. **14**:115. 1889, pro parte max.

As already stated under *S. arctica* Pall., the existence of this previous name had apparently been overlooked by BROWN in establishing his new *arctica*, of which RICHARDSON was the first to give a description. It may be that this diagnosis was prepared by BROWN, because RICHARDSON in his preface expressly acknowledges the great assistance BROWN gave him, but the first edition of RICHARDSON's Botanical Appendix appeared shortly before the Chloris Melvilliana, in which BROWN published an excellent description of his species. When in 1831 CHAMISSO changed BROWN's

name to *S. anglorum* after having given a good account of *S. arctica* Pall., non Br., he said nothing but the following:

> *Salix anglorum* N.—*S. arctica* R. Brown ex Ed. Nees 1. p. 406, supl. to the append. of Cap. Parry's Voy. p. 282. E. Meyer Lab. p. 32 (non Pallas).—Insula et sinus Sti. Laurentii.—Ex insula Chamissonis, capsulis maturis vetustate calvescentibus.

These specimens, however, do not belong to *S. anglorum*, but what in 1839 HOOKER (Fl. Bor.-Am. **2**:153) referred to *S. retusa*. HOOKER, therefore, quotes *S. anglorum* in his synonymy of this species, for which ANDERSSON (1858) proposed the name *S.* (*retusa**) *phlebophylla*, and made it a species (*S. phlebophylla*) in the Prodromus (1868). Here he also quotes *S. anglorum* in the synonymy, having in 1858 ignored entirely CHAMISSO's name, which has been used by some later authors for *phlebophylla* instead of *anglorum*. RYDBERG (1899) was the first to state that CHAMISSO's name "must be regarded as equivalent" to *S. arctica* Br.

What, however, is the typical *S. arctica* Brown? It was first collected by ROSS during his exploration of the "Baffin Bay, Lat. 70° 30′ to 76° 12′ on the east side, or at Possession Bay, Lat. 73°, on the west side." RICHARDSON probably based his description chiefly on his own plants collected on "barren grounds from Point Lake to Arctic Sea" (or, as the explanation in ed. 2 runs, on "barren grounds from Lat. 64° to the Arctic Sea, in Lat. 60°"); while BROWN, besides the plants of ROSS, mentioned those of PARRY's Expedition from Melville Island, Winter Harbor. I have not yet seen a type specimen, but RICHARDSON's and BROWN's descriptions are sufficient to furnish us with the following characters:

Frutex depressus. Rami, decumbentes, floriferi omnes et sterilium nonnulli adscendentes, adulti glabri. Folia elliptico-obovata vel obovata, integerrima, novella pilis sericeis vestita, adulta utrinque glabra, venis subtus parum eminentibus, venulis anastomosantibus. Amenta utriusque sexus ramos brevissimos foliatos terminantia. Squamae orbiculato-obovatae, saepe retusae, fusco-nigricantes pilis sericeis vestitae. Mascula 8–10 lin. longa, densa. Stamina 2, filamentis distinctis, antheris purpureis. Glandulae

duae. Ovaria sessilia vel brevissime pedicellata, dense griseo-tomentosa. Stylus longitudine varians, nunc stigma aequans, nunc fere dimidio brevior. Glandula unica.

Judging by these characters, there seems no doubt what form must be taken for the true *S. arctica* Br., that is, *S. anglorum* Cham. According to TRAUTVETTER (see under *S. arctica* Pall.) there have been distributed by HOOKER specimens under the name of *S. arctica* Br. which do not belong to this species, and TRAUTVETTER (1847) says that his *arctica* of 1832 (t. VI.) is not identical with BROWN'S plant. However, so far as I can judge by TRAUTVETTER'S diagnosis and figure, I believe that he had the true *S. anglorum* before him. Of course, only an inspection of his type can make a final decision regarding its identity possible. Of ANDERSSON'S treatment of *S. arctica* Br. I have already spoken. LUNDSTRÖM, who apparently misinterpreted the name *anglorum*, chose ANDERSSON'S (varietal) name *Brownei* for what he believed to be *S. arctica* Br. I strongly suspect that *S. Brownei* Ldstr. only partly belongs to *S. anglorum*, and an investigation of LUNDSTRÖM'S specimens from Nowaja Semlja is needed to decide what he really understood by his *S. Brownei*. It seems to me most unlikely that the true *S. anglorum* should at all occur on Nowaja Semlja or in Arctic Asia or Europe; and the description given by LUNDSTRÖM, in my opinion, does not fit BROWN'S species. There may be in Arctic Asia and Europe similar forms which, however, in reality belong to *S. arctica* Pall.

BEBB, as already stated, unfortunately did not know LUNDSTRÖM'S work when proposing a new *S. Brownii* which comprised *S. arctica* And. (1868) "excl. var. *nervosa*." He created a new mixture of forms, including *S. groenlandica*, *S. petrophila*, *S. taimyrensis*, and others. In April 1899 RYDBERG said: "There is scarcely a species that has been so misunderstood as this [*S. arctica* Br.]. Even Mr. BEBB, who cleared up somewhat the discrepancy between *S. arctica* Pall. and *S. arctica* Br., had a very vague idea about the latter." RYDBERG himself did not interpret correctly BROWN'S species. He quotes as type "Franklin Expedition, Dr. *Richardson*," and cites a specimen of the "Herb. Hooker, Barratt, and Torrey, no. 93," which I have before me and which bears the label

"*S. arctica*, Fort Franklin, Mackenzie River." It contains male and female branchlets with young flowers and very young, narrowly lanceolate, rather acute leaves. So far as I can judge by the thinly pubescent and distinctly pediceled ovaries, by the oblong bracts, and by the absence of a dorsal gland in the male flowers, the specimen does not belong to *S. anglorum*, but may probably be referable to *S. groenlandica* Ldstr. Furthermore, RYDBERG states that his *S. anglorum* "is characterized by . . . . the exceedingly large catkins, which are rather loosely flowered below, and the large conic capsule, which is only moderately hairy." If we compare this statement and the specimens cited by RYDBERG, his misinterpretation of BROWN'S species is evident. RYDBERG refers to his *S. anglorum* mostly specimens that in reality belong to *S. groenlandica*, about which species he certainly had a very wrong idea.

Almost simultaneously with RYDBERG (May 1899), BALL published a statement regarding *S. arctica* and BEBB'S treatment of this species. He knew LUNDSTRÖM'S study, but overlooked *S. anglorum* Cham.; he said, however, "I shall not rename the plant now, for I believe the name which has been in use for 80 years (*S. arctica* R. Br.) can yet do duty until both the numerous variations and the synonymy have been given careful study." In BRITTON and BROWN'S Ill. Fl. (ed. 2. 1:605, *fig. 1489*. 1913) the name *S. anglorum* is applied to forms from "Labrador to Alaska, and in the Rocky Mountains to Colorado" in a way I do not understand.

Judging by the ample material before me, *S. anglorum* seems more variable than *S. arctica*. The habitat of the northeast American plant ranges from Northwest Greenland (about Disco Island) and Labrador (where it apparently does not occur south of the 55th parallel) through northern Ungava along the Hudson Strait and the northern shores of the Hudson Bay to the Franklin Bay, reaching, as it seems, its most western point at Cape Bathurst and not ranging beyond 130° W. longitude. There are some forms collected on Herschel Island (coast of Yukon Territory) which might be taken for *S. anglorum*, but on account of the absence of stomata in the upper surface of the leaves I refer them to *S. arctica*. Between 130° and 140° W. longitude there may be the meeting ground for the 2 species, and we need much more and well collected material from

there to get a correct conception of the relationship of those arctic forms. I am not fully convinced that the presence of stomata in the leaf surface of *S. anglorum* and their absence in *S. arctica typica* can be regarded as a decisive character in distinguishing certain similar forms, but I think this specific character is of great taxonomic value at least in several species. A. and E.-C. CAMUS lay much stress upon this character in establishing their systematic arrangement according to anatomical features, and an excellent observer like the well known dendrologist E. KOEHNE was always inclined to pay much attention to those characters. In studying willows we should bear in mind the following remarks of the distinguished English salicologist, F. BUCHANAN WHITE (Jour. Linn. Soc. **27**:346 [Rev. Brit. Willows]. 1890):

> Whilst all the parts of the plant are variable, some characters, on which a great deal of reliance has been placed, are so inconstant that they may, in many cases at least, be almost or quite ignored, though in other instances they are really of importance. Familiarity with the species can alone teach the student what are the points on which he can depend.

At present it is impossible to interpret properly certain forms because we do not yet know the degree of variation of the species in question. There are, I am convinced, many hybrids, and the fact that has been recognized by all the leading salicologists in Europe "that willows hybridize with the greatest facility adds," as WHITE (*loc. cit.*, p. 340) says, "immeasurably to the intricacies of the study." Here in America we are only just beginning to get a better understanding of the taxonomy, variation, and distribution of the numerous willows, and everyone who attempts to further our knowledge of them ought to be lenient in his criticism of those interested in this study.

It is not without hesitation that I propose the following varieties of *S. anglorum*, but I am encouraged by the fact that such a keen observer as Professor M. L. FERNALD, who has collected most of the material of the new forms and to whom I wish to express my gratitude, agrees with my treatment of them.

S. ANGLORUM var. **kophophylla**,[4] nov. var.—Frutex prostratus ramis subterraneis ad ultra 1 cm. crassis, ramulis repentibus pl. m.

[4] The name is derived from κωφός, blunt.

elongatis, fructiferis ut videtur tantum ascendentibus; ramuli novelli sparse, rarius subdensius pilosi, vulgo citissime glabrescentes, in sicco nigrescentes vel flavescentes vel hornotini autumno ut annotini purpurascentes, ad 2 mm. crassi, annotini biennesque purpurei badiive, interdum ut vetustiores pl. m. pruinosi, vetusti crassiores pl. m. nigrescentes. Gemmae ovatae, obtusae, glabrae, badiae, saepe leviter pruinosae, ad circ. 5 mm. longae, floriferae ut videtur obovatae, obtusiores. Folia adulta satis chartacea, inferiora minora variabilia, superiora majora vulgo late ovalia, ovato-rotundata, obovata, late elliptica ad orbicularia, apice rotundata vel satis breviter acuta, interdum brevissime plicato-apiculata, basi late cuneata, rotundata ad subcordata, 1.5:1.2 vel 2.3:1.8 ad 3.5:2.5–2.8 cm. magna, interdum ovato-rotunda ad 3.5 cm. longa et 3 cm. lata, margine integerrima, rarius partim sparse subdenticulata, vulgo parce (juniora densius) ciliata, superne ut videtur tantum novella pl. m. sparse villosula et in costa pilosula, cito glabra, saturate et vivide viridia, stomatifera, costa subimpressa nervis lateralibus subprominulis et etiam graciliter reticulata, subtus valde discoloria, glaucescentia, pruinosa, initio magis quam superne sericeo-villosa sed etiam (infimis minoribus exceptis) cito glabra, costa nervisque primariis utrinque 5–8 pl. m. flavescentibus vel brunnescentibus elevato-nervata et graciliter sed distincte reticulata. Petioli longitudine satis variabiles, superne sulcati, initio pl. m. pilosi, dein glabri, 2–14 mm. longi. Stipulae nullae vel raro evolutae, lineari-lanceolatae, glanduloso-denticulatae, subglabrae, visae vix ultra 2 mm. longae. Amenta satis serotina, ramulos foliatos 0.5–2(–2.5) cm. longos pl. m. pilosos terminatia, cylindrica, rhachi villosa; mascula (no. 3232) 1–2 cm. longa et circ. 8 mm. crassa; bracteae obovato-oblongae ad late obovatae, apice obtusae vel retusae, omnino fuscae vel apicem versus atrae (in vivo pl. m. purpurascentes?), utrinque satis longe sericeo-pilosae; stamina 2; filamenta libera, glabra, bracteis demum duplo longiora; antherae parvae, ellipsoideae, ut videtur violaceae; glandulae 2 (vel interdum 1), ventralis ovato-rectangularis, truncata, integra (semper?), bractea duplo brevior, dorsalis (3232) duplo minor et angustior (in no. 510 nulla); amenta feminea sub anthesi ut videtur circ. 1–1.5 cm. longa et 0.6 cm. crassa, satis densiflora, fructifera

ad 3.5:1.4 cm. magna; bracteae ut in floribus masculis; ovaria sub anthesi ovoideo-oblonga ellipticave, sessilia vel subsessilia, albo- vel griseo-villoso-tomentosa; styli distincti, vulgo apice bifidi, rarius subbipartiti, stigmatibus oblongis bifidis 2–2½plo longiores; glandula 1 ventralis, anguste ovato-conica, truncata, integra vel ut videtur pleraque bifida bipartitave, bractea subduplo brevior fructus elliptico-conici, circ. 7 mm. longi, fere sessiles, ut ovaria vel minus dense pilosa (interdum in forma porro observanda [nos. 61 et 62] fere glabri), valvis apertis paullo recurvatis.

TYPE LOCALITY.—Western New Foundland, Bay of Islands, northeastern region of the Blomidon Mountain.

RANGE.—Western New Foundland, Bay of Islands and Bonne Bay, and western Gaspé Peninsula, Mt. Albert.

SPECIMENS EXAMINED.—Western New Foundland, northeastern region of the Blomidon ("Blow-me-down") Mountains, serpentine tableland, alt. about 550 m., July 24, 1910, *Fernald* and *Wiegand* (no. 3231, f.; G.; "prostrate near melting snow"; no. 3232, m., 3233, fr. type; G.; "prostrate"); Blomidon Range, July 3–5, 1911, *C. S. Stewart* (no. 29, st.; G.); Bonne Bay, serpentine tableland, alt. about 380 m., August 27, 1910, *Fernald* and *Wiegand* (nos. 3227, 3228, fr.; G.)—Gaspé Peninsula, Mt. Albert, deep ravine near snow, July 23, 1881, *J. A. Allen* (m., f.; G.); north slope of Allen's ravine, on hornblende schist, July 26, 1906, *Fernald* and *Collins* (nos. 501, 503[b], f., 507, fr.; G.); on wet serpentine slopes, July 23, 1906, *Fernald* and *Collins* (nos. 508, f., 510, m., 514, fr.; G.); brookside near permanent water, alt. 700 m., August 13, 1905, *Collins* and *Fernald* (no. 60, fr.; G., N.; partim fructibus satis glabrescentibus); dry serpentine barrens, 1000–1050 m. alt., August 9, 1905, *Collins* and *Fernald* (no. 62, fr.; G.; partim fructibus glabratis ut in no. 60).

In its rather short and dense catkins, at the base not or hardly loosely flowered, this variety approaches typical *S. anglorum*, but differs in its firmer, more rounded, and soon glabrous leaves and the glabrate twigs, in which characters it comes near to the following varieties. There is also no. 3235 of *Fernald* and *Wiegand*, collected at the same time as no. 3231 "from near sea level to serpentine tableland, alt. about 550 m." as a prostrate shrub. It occurs, according to the specimens in Herb. G., with the rounded leaves of the typical *kophophylla*, and also with more acute, lanceolate leaves, and both forms seem not to possess stomata in the upper leaf surface. So far as we know at present, there is no *S. cordifolia* in the Blomidon Mountains, and therefore this form cannot be connected in any way with *cordifolia* var. *Macounii* (Rydbg.) m. (see my second article). It certainly needs further observation. No. 3234, collected by *Fernald* and *Wiegand* in the northeastern region of the Blomidon Range, on serpentine tableland, about 550 m. alt., July 24, 1910

(fr.; G.), much resembles *S. cordifolia*, but I found stomata in the upper side of the leaves. We do not know enough of the willows of this range to be able to determine this form properly.

S. ANGLORUM var. **araioclada**,[5] nov. var.—Frutex ut sub var. *kophophylla* descriptus sed sequentibus signis distinctus: folia adulta satis tenuiter papyracea, minora inferiora obovalia, obovato-oblonga vel ut majora superiora ovalia, elliptica, ovato-elliptica, obovato-elliptica vel rarius obovato-lanceolata, apice vulgo magis obtusa vel rotunda quam acuta, raro retusa vel subito plicato-acutata, basi rotundata ad late cuneata, rarius sensim attenuata, margine integerrima, minimis exceptis 1.5:1 vel 3:2 ad 4:2.7 vel 5:2.9 cm. vel angustiora acutiora ad 4:1.8 cm. magna, superiora vulgo ab initio glaberrima; petioli interdum ad 10 mm. longi; stipulae rarissime evolutae, minimae, lineari-lanceolatae, caducae; amenta fructifera ramulos foliatos ad 4 cm. longos terminantia; mascula 1.5–2.5:1 cm. magna, minus quam in typo sericea; bracteae longe sed satis laxe sericeae; glandulae 2–1; feminea sub anthesi ad 3.5:1 cm. magna, fructifera vulgo 3.5–5.5 cm. longa et 1.8 cm. crassa, basim versus vulgo distincte laxiflora; bracteae interdum quam in masculis oblongiores sed saepissime densius sericeae, extus ad apicem interdum partim glabrescentes; ovaria ovoideo-oblonga, griseo-villoso-tomentosa, sessilia vel subsessilia; styli distincti, integri vel apice breviter bifidi, quam stigmata oblonga bifida vix duplo (rarius in floribus valde juvenilibus fere $2\frac{1}{2}$plo) longiores; glandula ut in typo longa, pl. m. anguste conica, bractea duplo brevior; fructus ovato-conici, maturi ad 7–8 mm. longi pedicello subnullo vel brevi glandula $\frac{1}{3}$ ad 2plo breviore excluso, laxius quam ovaria villoso-tomentosi, fulvi.

TYPE LOCALITY.—Gaspé Peninsula, north slope of Mt. Albert.

RANGE.—Gaspé Peninsula and the Selkirks and Rocky Mountains in British Columbia, Asulkan Valley, and in Alberta, near Laggan and Jasper Park.

SPECIMENS EXAMINED.—CANADA: Quebec, Gaspé County, Mt. Albert, deep ravine near snow, alt. 810 m., August 2, 1881, *J. A. Allen* (m.; G.; amentis parvis ovatis, glandula dorsali nulla, forma porro observanda); alt. 750–1050 m., July 26, August 2, 1881, *J. A. Allen* (m., f.; G. ex herb. Bebb;

[5] Derived from ἀραιός, slender, and κλάδος, branch.

amentis parvis); head of Allen's ravine, August 8–15, 1905, *Collins* and *Fernald* (fr. im.; G.); north slope of same mountain, on hornblende schist, July 26, 1906, *Fernald* and *Collins* (no. 500, m. paratype; G.; 501[a], f.; G.; 503, 503[a], f. adult., 505, f. type; G.); July 20, 1906, *Fernald* and *Collins* (no. 506, fr.; G.); on wet serpentine slopes, July 23, 1906, *Fernald* and *Collins* (no. 510[a], m.; 514, fr.; G.).—BRITISH COLUMBIA: Selkirk Mountains, Rogers Pass, alt. 1350 m., July 31, 1890, *J. Macoun* (no. 18[a], f.; N.); Asulkan Valley, Glacier, alt. 1590 m., *J. G. Jack*, August 14, 1904 (fr.; A., G.); same place and date, *A. Rehder* (fr.; A.; both specimens identical with those like no. 506 from Gaspé).—ALBERTA: Lake Agnes near Laggan, August 11, 1904, *A. Rehder* (m., f.; A.; forma incerta quamvis ad *S. petrophilam* spectans); slopes of ravine on Mt. Aylmer, alt. 2250 m., August 4, 1899, *W. C. McCalla* (no. 2248, m., f.; Cor.); mountains above Lake Louise, alt. 1800–2400 m., July 21, 1907, *F. K. Butters* and *E. W. D. Holway* (no. 262, f.; N.; forma quasi ad *S. petrophilam* transiens sed foliis magis quam in hac specie discoloribus); Lake Louise, July 22, 1904, *J. Macoun* (no. 68883 O., fr.; N.); Fitzhugh Mountain, near Jasper Park, August 1917, *J. M. Macoun* (nos. 95379, 95397, 95398, 95401 O., fr., m.).

This peculiar variety differs from the type chiefly in its less pubescent, mostly much more elongated, and yellowish twigs, in its almost glabrous young leaves, and in its aments which, on an average, are longer and thinner, at least much more loosely flowered toward the base. It is, apparently, closely connected with var. *kophophylla*, which as a whole has firmer leaves and denser and shorter catkins, but in its glabrous character comes nearer to var. *araioclada* than to the typical *anglorum*. See also my remarks under the following form.

S. ANGLORUM var. **antiplasta,**[6] nov. var.—Frutex habitu ramulisque ut in var. *araioclada;* folia adulta chartacea, anguste ovalia, elliptico-oblonga, anguste obovato-oblonga, interdum oblanceolata, rarius elliptica vel obovato-elliptica, utrinque pl. m. acuta, raro rotundata, saepe apice breviter plicato-acuminata, vulgo 1.5–2.5 cm. longa et vix ultra 1 cm. lata, maxima ad 3:1.3–1.5 (rarius 1.8) cm. magna, integerrima vel interdum basim versus obsolete parce denticulata, superne subtusque ut in var. *araioclada* sed nervis lateralibus vulgo ut in *petrophila* angulo acutiore a costa abeuntibus et magis versus apicem currentibus; petioli graciles, 2–8 mm. longi, vulgo sparse pilosi; amenta cylindrica, sub anthesi satis brevia et tenuia, vulgo sublaxiflora, ramulos laterales in masculis vix ad 1 cm. longos ceterum ut in *araioclada* terminantia,

[6] Derived from ἀντίπλαστος, similar.

rhachi parteque nudo pedunculi pl. m. villosa; mascula vix ad 1.5:0.7 cm. magna, bracteae et cetera ut in *araioclada*, glandula dorsalis (an semper?) nulla; feminea sub anthesi 1–2:0.5–0.7 cm. magna, fructifera vix ad 3 cm. longa et 1.2 cm. crassa, bracteae ut in masculis; ovaria ovoideo-oblonga, pl. m. sessilia; styli distincti, saepe apice breviter bifidi, stigmatibus brevibus oblongisve paullo vel ad 2.5plo longiores, glandula ut in varietate precedente; fructus ovato-conici, subsessiles, ad 6 mm. longi, laxius quam ovaria villoso-tomentosi vel anni praeteriti subglabrescentes.

TYPE LOCALITY.—Gaspé Peninsula, serpentine slopes of Mt. Albert.

RANGE.—As above.

SPECIMENS EXAMINED.—CANADA: Quebec, Gaspé Peninsula, Mt. Albert, serpentine slopes, July 23, 1906, *M. L. Fernald* and *J. F. Collins* (no. 509, f., fr., type; G.); exposed serpentine barrens, alt. 1000 m., August 9, 1905, *Collins* and *Fernald* (no. 61, m., f.; G., N., O.); sheltered mossy knolls, August 10, 1905, *Collins* and *Fernald* (no. 61ª, f.; G., N., O.; a precedente nonnisi petiolis vulgo longioribus differe videtur); on wet serpentine slopes, July 23, 1906, *Fernald* and *Collins* (no. 511, fr.; G.; forma gracilis juvenilis, habitu *S. petrophilae* valde similis); north slope of same mountain, on hornblende schist, July 26, 1906, *Fernald* and *Collins* (no. 504, f. defl.; G.; forma satis vegeta, ramulis elongatis, foliis pl. m. plicato-acuminatis).

At first sight this variety much resembles *S. petrophila* in its habit, the shape of the leaves, and the yellowish color of the young twigs, but the leaves are of a deeper green on the upper surface and much paler and glaucescent on the lower surface, and do not differ in this respect from any other form of *S. anglorum*. It is, however, much easier to distinguish herbarium specimens of both species than to express the differences in exact words. The two species meet each other in the Rockies of Alberta and British Columbia, and there are also certain forms in northern Montana, and even in Wyoming which at present I am at a loss to determine. Some of them may represent hybrids between *S. petrophila* and other species with which I am not yet sufficiently acquainted.

3. S. PETROPHILA Rydbg., in Bull. N.Y. Bot. Gard. **1**:268. 1899, is the species which seems to be nearest related to *S. anglorum*. It was first described by ANDERSSON (DC., Prodr. **16**²:287. 1868) as *S. arctica petraea* from specimens collected by *E. Bourgeau* "in summo Rocky Mountains." I have seen a photograph of the type at Kew and a cotype in the Gray herbarium. Both specimens bear the label of PALLISER'S Brit. N. Am. Expl. Expedition, with the printed indication "Rocky Mountains" and "coll. E. Bourgeau

1858"; and upon them is written "*Salix arctica* R. Br. *subalpestris* And. (forte n. sp.)." ANDERSSON apparently changed the varietal name later to *petraea*. The Kew sheet also bears, in the lower left corner, the inscription "*Salix herbacea*. Montagnes rocheuses Palouse près les Glaciers. 18 août 1858." According to MACOUN (Cat. Canad. Pl. preface, p. viii. 1883), BOURGEAU "spent some time, in August 1858, in the Bow River Pass and the adjacent mountains" in Alberta. *S. petrophila* differs from *S. anglorum* chiefly in the color of the rather pale or grayish green leaves, which are not distinctly paler and never whitish beneath. The differences indicated by RYDBERG between the two species are of no value, because his *S. anglorum* is mostly *S. groenlandica*. As I have already said, there are some forms in the northern habitat of *petrophila* which I have not yet been able to interpret properly. So far as I can judge by the specimens before me, the species ranges from about 52° N. latitude in southwestern Alberta and southeastern British Columbia through western Montana, northeastern Wyoming, and central Colorado to the Truchas Peak in northern New Mexico. I have not seen specimens from Washington and it is not mentioned in PIPER'S Flora. In eastern Oregon I know only of two localities. From Utah and Nevada I have seen very little material, and in California it is found in the Sierra Nevada from Sierra County to Tulare County.

In western Nevada and the Californian Sierra, *S. petrophila* is mostly represented by a form which has been described as *S. caespitosa* by KENNEDY (Muhlenbergia **7**:135, *pl. 9*. 1912). Through the kindness of Professor C. W. LANTZ I have seen the type, which is preserved in the herbarium of the Agricultural Experiment Station at Reno. It was collected by the author on Mount Rose, Washoe County, Nevada, August 17, 1905 (no. 1173, fr.). It differs from typical *petrophila* in the more copious pubescence of the upper leaf surface, the acuter leaves, and the very short style. The last character seems very variable, and the type material before me consists only of fruits with withered styles and stigmas. Nevertheless, I am inclined to use the name *caespitosa* for a variety which seems to be the prevailing form in the western part of the range of *petrophila*, and this var. **caespitosa** (Kennedy), nov. var., may be

distinguished by its foliis utrinque acutioribus apice subacuminatis superioribus superne (saltem in parte) satis villosis, subtus vulgo glabris ad 3.5:1.3 cm. magnis, amentis femineis (immaturis) interdum ad 6:1.3 cm. magnis basi valde laxifloris longe pedunculatis. The most extreme form of this variety has been collected by *Hall* and *Chandler* on Mount Goddard, Fresno County, California, July 24–26, 1900 (no. 685, m., f.; G.); and I refer to it also a specimen collected by *F. W. Congdon* on Mount Dana, Mono County, California, August 27, 1895 (m., fr.; N.).

It may be mentioned here that *S. cascadensis* Cock. (*S. tenera* And., non A. Br.) is regarded as very closely related to *petrophila* by RYDBERG, or as "perhaps only a variety" of it by BALL. I prefer to place it in a different group next to *S. phlebophylla*, and I shall speak of it later.

There are three more willows, which, in my opinion, should be included in the same group with *S. arctica*, namely *S. stolonifera* Cov., *S. ovalifolia* Trautv., and *S. groenlandica* Ldstr. The first two have been well treated by COVILLE (1901), and need only a few remarks, while the history and taxonomy of the last ought to be explained in detail.

4. S. STOLONIFERA Coville, in Proc. Wash. Acad. Sci. **3**:333. *pl. 41. fig. 1* (Willows of Alaska). 1901, "is a species of eastern Alaska, in the glacier region from Yakutat Bay to Glacier Bay and Lynn Canal." RYDBERG (1899) mentioned this species under the name of *S. unalaschensis* "Cham. Linnaea **6**:539." As COVILLE has explained, CHAMISSO did not propose such a species, but merely describes a "*Salix* unalaschcensis, multis cum *arctica* Pall. conveniens, pluribus ab illa abhorrens, nulli nostrarum propius accedens," to which he did not give a specific name. His form from Unalaska is the same as *S. ovalifolia* Trvt., and ANDERSSON has already mentioned in the Prodromus "*S. unalaschkensis* Chamisso" among the synonyms of TRAUTVETTER'S species. COVILLE describes the ovaries as "smooth or with some traces of pubescence toward the apex," and he regards the glabrous form as the typical and common one. I think it best to propose a f. **subpilosa,** f. nov., fructibus pl. m. interdum satis dense pilosis, because such forms resemble somewhat *S. arctica*, especially when the old fruits have lost the

style. The leaves, so far as I can see, always possess stomata in the upper epidermis, as is the case with typical *S. stolonifera*, while they are wanting in the leaves of typical *S. arctica* and *S. ovalifolia*. The length of the style and the rather long linear stigmas seem to be the best characters to distinguish *S. stolonifera* from the other species of this group. "The characteristic of the production of slender leafless, subterranean branches or stolons" is not always clearly seen on herbarium specimens, and the presence of such stolons may possibly be detected in other related species.

5. S. OVALIFOLIA Trautvetter in Nouv. Mém. Soc. Nat. Mosc. **2**:306, *pl. 13* (De Salic. Frig. Kochii). 1832.—*S. myrtilloides* forma 4 Chamisso in Linnaea **6**:539. 1831.—*S. unalaschkensis* Chamisso ex Andersson in Öfv. K. Vet.-Akad. Förh. **15**:130. 1858.—*S. rotundata* Rydberg apud Macoun, List Pl. Pribilof Islands in Jordan, Fur Seals N. Pac. **3**:571. 1899, non Forbes 1829.—*S. cyclophylla* Rydberg in Bull. N.Y. Bot. Gard. **1**:275. 1899, non Gandoger 1882.—The type locality of the species is Cape Espenberg in the Kotzebue Sound. Its range extends from the Bering Strait, where it is probably also found on the Siberian Coast,[7] northward to Point Barrow and Martin Point, where it has been found by *F. Johansen*, July 30, 1914 (no. 136[b] or 93484 O., fr.); and southward to the Pribilof and Aleutian Islands and the Alaskan Peninsula, but it has also been collected on Kodiak Island, and to the eastward as far as Yakutat Bay. The typical form has glabrous ovaries and fruits; there are, however, specimens with loosely pubescent capsules collected by *Trelease* and *Saunders*, St. Paul Island (no. 3442, fr.; M.), which may represent the var. *pubescens* And. (DC. Prodr. **16**[2]:291. 1868). This is described as being distinguished by "capsulis tenuiter hirsutis griseo-pubescentibus petiolis et foliis basi longius hirsutis." As no type is given, I cannot decide whether ANDERSSON'S variety is identical with this specimen.

Some other specimens which COVILLE has cited as typical *S. arctica*, while RYDBERG took them for *S. diplodictya* Trautv., should be discussed. The last species has been described, as I have explained, as having the leaves green and glossy on both sides, and

[7] Lg. C. WRIGHT in 1853–56 on Arakam Island. Those specimens are distributed as *S. uva-ursi*, but agree well with *S. ovalifolia* except that the fruits are not glaucous.

it has certainly nothing in common with the forms in question. These specimens seem to represent a form somewhat intermediate between typical *S. ovalifolia* and typical *S. arctica*. It may be characterized briefly as follows: ab *ovalifolia* satis diferre videtur foliis amentisque majoribus, floribus masculis tantum (an semper?) glandula ventrali instructis, ovariis satis pubescentibus etiam fructibus tenuiter vel partim (fere ut in var. *pubescente* supra) pilosis sed non distincte glaucescentibus; ab *arctica* praecipue recedit foliis minoribus pl. m. rotundatis vel obovato-rotundis, amentis parvioribus, fructibus minoribus (perfecte maturis non visis) pl. m. glabrescentibus vel partim glabris. I do not want to propose a new name for this form, because it needs further observation, but it is by no means identical either with *S. ovalifolia pubescens* or with *arctica*. It may be referred provisionally to *S. ovalifolia* var. *subarctica* Lundström in Nov. Act. Roy. Soc. Sci. Upsala III. 1877. p. 41, where the following characters are given: "*β, subarctica* nob. capsulis pubescentibus; foliis majoribus, subtus parce villosis." As I have said, the forms described by LUNDSTRÖM cannot be fully understood until his type material is examined.

There remains another arctic form which I should have regarded as not separable from typical *S. ovalifolia* but for the fact that I found stomata in the upper leaf epidermis in most of the specimens cited later. So far as I can judge by the rather scanty material before me, this variety, for which I propose the name var. **camdensis,** var. nov., seems chiefly to differ from *S. ovalifolia* in the following respects: foliis nondum perfecte evolutis minoribus vel oblongioribus elliptico- vel ovato-oblongis vel oblanceolatis apice acutis vel obtusis basi acutis vel pl. m. rotundatis vix ultra 1.5 cm. longis et 1 cm. latis in epidermide superiore vulgo pl. m. stomatiferis adultis textura tenuiore et subtus minus distincte reticulatis, petiolis saepe quam gemma brevioribus, amentis masculis submajoribus ad 1.5:1 cm. magnis, fructiferis subminoribus ad 1.5 cm. longis et 1.2 cm. crassis.

I examined the following specimens: Alaska, Camden Bay, Collinson Point, July 17, 1914, *F. Johansen* (no. 116 or 93482 O., fr., type in O.); June 1914, *F. Johansen* (no. 44[a] or 93807 O.; f.; stomata non visa; no. 44[b] or 93806 O., m.); Kongenevik, July 1914, *F. Johansen* (no. 82[a] or 93805 O., m.

syntype; no. 82[b] or 93804 O., fr.; stomata superne in foliis non visa); west of Martin Point, July 30, 1914, *F. Johansen* (no. 136[a] or 93483 O., st.; folia superne stomatibus numerosis instructa breviter petiolata, forma porro observanda).

Dr. FRITS JOHANSEN has been so kind as to give me the following information regarding this variety: "Nos. 44a, b, Collinson Point. This willow grew on more bare, gravelly tundra near the beach (transition region to the latter), in patches of several plants. Its growth was very prostrate and depressed (among stones and vegetation), with the stems and branches lying very close to the ground and spreading widely, so that only the catkins showed up from a little distance. Especially the subterraneous parts (roots and stem parts) were less extensive and spreading than with those found at Kongenevik, Alaska (see below); probably because they did not grow on sand dunes as is the case at the former place.—Nos. 82a, b, Kongenevik. The collecting place was where the seashore (beach) through low sand dunes goes over into the more typical tundra behind. On these sand dunes the vegetation is very characteristic and consists almost exclusively of *Elymus*, *Carex*, *Salix*, *Chamaerium*, etc.; each species spreading (both above and under the ground) over large patches (areas) and dominating more or less to the exclusion of the other species. This *Salix* seemed to be very prostrate, but the larger part of each plant is buried in the sand, so that only the leaf and catkin-carrying branch parts (outer third) protruded. It was mostly large plants widely spreading (both roots and stems); the branches often having the form of long "runners" intersecting the sand rhizome-like in all directions. The sand-covered parts of the branches were without leaves or catkins and pale (white-yellow). When growing in less sandy soil the growth is naturally more condensed (see above under Collinson Point). The plants were in full bloom in the end of June.—Nos. 136a, b, Martin Point. The collecting place was a sandy gravel spit of slight elevation, with the sand dunes less pronounced than at Kongenevik. Vegetation rather scattered and in patches, except around the several ponds and the big lagoon between the sand spit and the mainland behind. On sandy places the vegetation was much like that at Kongenevik, with *Honckenyia* taking the place of *Chamaerium*. As the character of the spit was somewhat intermediate between the beach regions at Collinson Point and at Kongenevik, so did also the growth of the *Salix* in question resemble those of the same species from both of the foregoing places. At the time of collecting the plants had dropped ♂ catkins and had unripe ♀ catkins."

6. S. GROENLANDICA Lundström, Nov. Act. Reg. Soc. Sci. Upsala III. 1877. p. 36.—*S. arctica* Liebmann, Fl. Dan. XIV. fasc. 42:7, *pl. 2488*. 1849, non Pall.—*S. arctica* γ, *Groenlandica* And. in DC., Prodr. **16**[2]:287. ut videtur excl. forma 6 *pusilla*.—ANDERSSON

based his var. *groenlandica* on "*S. arctica* Fl. Dan. t. 2488," and he distinguished 6 forms: (1) *hebecarpa*, which is nothing but the type; (2) *lejocarpa*, with glabrous ovaries; (3) *latifolia*, which probably only represents a vigorous form with "foliis orbiculato-ovalibus"; (4) *angustifolia*, a mere form with "foliis lanceolatis"; (5) *macrocarpa*, which is nothing but the typical plant with normal big aments; and (6) *pusilla*, which I cannot interpret because the description ("fruticulus vix digitalis, foliis 1–3 lin. longis densissime confertis. *Salici retusae serpyllifoliae* analoga") is insufficient, and ANDERSSON does not cite a type or any locality for it. The description and figure given by LIEBMANN are quite sufficient to understand what form is meant, and it is rather surprising that this well marked species could be misunderstood by later authors. LUNDSTRÖM did not say much about it, because he was dealing with Asiatic and European forms, and only wanted to separate it from the related species. LANGE (Consp. Fl. Groenl. 1:108. 1880), in adding his var. "*minutifolia* And. mscr." to those already described by ANDERSSON (but omitting f. *macrocarpa*), referred *S. arctica* Br. (*S. Brownei* Ldstr.) as a synonym to *S. groenlandica*, and seems to have misunderstood BROWN's plant. RYDBERG, in his turn, as I have said, mixed the real *S. groenlandica* with his *S. anglorum*, and gave the name *groenlandica* to specimens of the latter species and to several forms of different origin. In my opinion the true *S. groenlandica* may easily be recognized by its glabrous leaves, which are shining dark green and without stomata above and distinctly glaucescent beneath, the margin being entire or often more or less glandular denticulate, by its large aments which measure from 5:1.2 to 10:1.6 cm. in fruit, and by its distinctly pediceled ovaries, which bear a rather thin and short silky pubescence even when young and possess a short and broad gland of about half the length of the pedicel. The shape of the ventral gland, which is the same in both sexes, differs much from that of the other species of this group where, as a rule, it is oblong or ovate-conical and longer than the pedicel. The thin pubescence of the ovaries and fruits, which are often almost glabrate or entirely glabrous in var. *lejocarpa* (And.) Lange, gives them a different aspect from the tomentose capsules of *S. arctica*, *S. anglorum*, or

S. petrophila. In the size of the fruiting aments *S. groenlandica* is next to vigorous forms of *S. arctica* and to *S. arctica* var. *subcordata*.

The type of *S. groenlandica* has been collected by VAHL "in locis humidis Groenlandiae orientalis et occidentalis a limite maris ad alt. 200 pedum." I have not seen a specimen of VAHL's and no material from eastern Greenland. Judging by the specimens I have examined, its range extends from Disco Island (70° N. latitude) through the southern part of Baffin's Land westward to the Bathurst Inlet (about 109° W. longitude), and southward along the shores of the Hudson Bay through Ungava and Labrador to the western Gaspé Peninsula and the Port à Port Bay in western Newfoundland. There are also some rather uncertain and fragmentary specimens from the Lancaster and Jones Sound, and probably the habitat of *S. groenlandica* reaches its northern limit at about the 76th parallel. Other specimens have stomata in the upper epidermis of their leaves and may represent a different variety or be of hybrid origin; they need further observation.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

FECUNDATION AND FORMATION OF THE PRIMARY ENDOSPERM NUCLEUS IN CERTAIN LILIACEAE

MILDRED NOTHNAGEL

(WITH PLATES III–V)

Introduction

Between 1890 and 1902 many articles appeared on fecundation and double fertilization in the angiosperms. On the whole, the authors have dealt with the entrance of the male nuclei, their behavior within the embryo sac, their form, the union between the egg and male nucleus, and between the male and polar nuclei; but they have not investigated in detail the chromatin changes that occur from the time of contact of these nuclei to the completion of the first division.

In 1891 GUIGNARD (**6**) described the entrance of the so-called antherozoids into the sac, each accompanied by its centrosomes. One male nucleus became applied to the egg nucleus, each of which took on the resting condition and remained distinct for some time. While in this state the male nucleus enlarged and both the egg and the sperm nucleus flattened at the surface of contact, but with a distinct line of demarcation remaining between them for some time. Even after the nuclear membranes had disappeared, the contour of the two was traceable at the periphery. Later he distinguished, on opposite sides of the nuclear cavity, two groups of chromatin in the spirem stage. No drawings were made to show this. When the nuclear plate was formed, he asserted that one-half of the chromosomes were contributed by the egg and one-half by the sperm.

The process of fertilization in *Lilium Martagon* and *L. candidum* was described by MOTTIER (**13**) in 1898. In *L. Martagon* there was no complete fusion of egg and S-shaped sperm, the lack of which resulted in a failure to mature seeds. In the region of the two polar nuclei, which had not fused and which began disintegrating 96 hours after pollination, a nucleus, similar to the nucleus which united with

the egg, was observed. *L. candidum* furnished material for normal fertilization. At the union of the egg and sperm, the latter was about the size of the former and both were in the resting condition, the chromatin being distributed in the form of a fine network. No boundary was observed separating the two elements at the point of contact, and the fusion that took place during resting condition was so complete at the close of fertilization that there was no visible distinction between male and female chromatin.

In 1904 MOTTIER (**14**) confirmed his earlier investigations, pointed out the S shape of the male nucleus, the fusion of the sexual nuclei in resting condition, the coming together of the two polar and male nuclei in *L. Martagon*, and the cause of the non-fusion. Although he stated that the sexual nuclei were in the resting condition at the time of fusion, he called attention to the chromatin of the sperm being more regular than that of the egg. It was also claimed that the nucleoli fused at fertilization.

One of the first reports of double fertilization was made by GUIGNARD (**7**) in 1899 for *Lilium Martagon*. In this species he observed the union of one of the male nuclei with one of the polar nuclei, followed by the union with the second polar nucleus. The chromatin of the two male nuclei, on account of being coarser, was distinguishable from that of the egg and polar nuclei with which they had fused. He also stated that he was able to recognize the triple origin of the secondary nucleus during the prophases, although no drawings were given.

NAWASCHIN (**15**), in the first report of double fertilization in *Lilium Martagon* and *Fritillaria tenella*, noted that the cellulose membrane surrounding the sexual apparatus was absorbed just previous to the entrance of the pollen tube, and that the spiral-shaped male nucleus entered the protoplasm of the sac. He concluded that the sperms took on various shapes under various conditions, and, as GUIGNARD had assumed, that they were motile. One sperm was found to enter the egg, the other to unite with the superior polar nucleus, and in both cases a complete fusion occurred after a certain period. The fusion of the superior and inferior polar nuclei took place after the male nucleus had united with the former. The triple fusion was followed in a short time by a division which preceded that of the egg.

In 1900 GUIGNARD (**9**) found that in some cases the polar nuclei, the upper one of which he said was analogous to the egg, fused before the entrance of the pollen tube, and that when the male nuclei entered the sac, they entered into fusion so quickly that, in some species, one rarely saw them free. In *Tulipa* he was able to follow the contour of the three nuclei entering into the fusion nucleus some time after their coming together, and even after the membranes had disappeared at their surface of contact.

As a result of investigations in various groups of angiosperms by GUIGNARD (**6, 7, 8, 9, 10**), NAWASCHIN (**15**), STRASBURGER (**17, 18**), and others at this time, it was generally concluded that double fecundation was normally found in angiosperms and that the uniting of the male nucleus with the polar nuclei was in the nature of a pseudo-fecundation whose function was to stimulate the formation of "albumen."

ERNST (**3**), investigating fertilization of *Paris quadrifolia* and *Trillium grandiflorum*, found that the two polar nuclei were fused before the male nucleus united with them, and at times a spirem was formed previous to the entrance of the sperm, showing that the male nucleus was not necessary to stimulate division. At other times the spirem was not formed until the three nuclei were fused, in which case he was unable to discern which part of the chromatin was contributed by the various nuclei. He also stated that it was not safe to rely upon the number of nucleoli found in the fused mass to ascertain whether fertilization had taken place or not. In the fecundation of the egg there was a complete blending of the substances, and at cross segmentation he failed to find the arrangement of the chromatin into two groups.

STRASBURGER (**17, 18**) used the terms generative and vegetative fertilization, the latter being applied to the triple fusion. The union of the sperm, either with the egg or with the polar nuclei, functioned as a stimulus.

In 1911 COULTER (**2**), after reviewing the literature on endosperm formation, stated that since endosperm may form without the fusion of the sperm or even of the second polar nucleus, these being simply supplementary, there seemed to be no reason why "there should be any hesitation in recognizing the endosperm as gametophyte." He concluded that "the product of such fusions is

merely an undifferentiated tissue which practically continues the tissue of the gametophyte, that is, it is simply growth and not organization."

From 1902 to 1913 practically nothing new was published on the subject of fertilization. In 1913 BLACKMAN and WELSFORD (1) reported that the chromatin of the vermiform male nucleus was in a network, although not the network of a resting nucleus, this condition becoming more noticeable later on. At times they also noted that the chromatin of the egg might become threadlike just previous to fusion.

The most recent paper on fertilization is by SAX (**16**) in 1916 on fertilization in *Fritillaria pudica*, in which he noted that the vermiform sperm lay indented in the egg for some time before the membranes between them disappeared. The chromatin was in more or less of a network and the granules were of various sizes. When the membranes at the surface of contact broke down, the contents of the two nuclei mingled and were not distinguishable from each other. The spirem usually appears after this. Triple fusion was also complete and the resulting nucleus divided before that of the fertilized egg.

In none of these cases have the chromatin changes been carefully followed from the time of contact of the nuclei until the completion of the first division, the emphasis previously having been placed upon the actual coming together, the uniting, and the very earliest steps in division.

The process of fertilization and distribution of the chromatin contributed by the egg and sperm in *Pinus* and *Abies* has been carefully worked out by FERGUSON (**4, 5**) and HUTCHINSON (**12**), and it was with the desire that something of this nature should be done for angiosperms that the present investigation was undertaken.

Materials and methods

For fertilization of the egg, *Trillium grandiflorum* was used, the material being collected in damp woods along the Des Plaines River, northwest of Evanston, Illinois, from May 3 to May 26, 1916. The first collection was made at the time of pollination, although the pollen tube was not seen in the micropyle until two weeks later,

while in the collection of May 26 dividing endosperm nuclei and second division of the fertilized egg were found for the first time. *Lilium Martagon*, collected from the garden of Indiana University, Bloomington, Indiana, in May 1916, 96 and 120 hours after pollination, was more favorable for the first division of the endosperm nucleus. The former material was killed and fixed in chrom-osmic-acetic acid 1–2 hours and then in chromo-acetic acid 24–36 hours, washed, dehydrated, and imbedded either from chloroform or xylol. *Lilium Martagon* ovaries were killed and fixed in chrom-osmic-acetic acid 24–36 hours, washed, dehydrated, and imbedded from chloroform. All sections were cut 12 μ thick and both modified triple and Heidenhain's iron-alum-haematoxylin were used for staining, the latter being more satisfactory for most stages, as the chromatin was more sharply differentiated.

Formation of the primary endosperm nucleus

For the development of the spirem and the first division of the endosperm nucleus, *Lilium Martagon* was found to be very favorable, as many dividing primary endosperm nuclei were found in the sacs of material killed 96 and 120 hours after pollination. Activity did not cease at the end of the first divisions, for as many as 12–16 nuclei were found in many sacs of the older material (fig. 29).

The sperm comes in contact with the polar nuclei before these two have fused, although they may be in contact or in close proximity (fig. 17). These three nuclei will usually be found in the center of the sac where just previous to the triple fusion the two polar nuclei were to be seen.

The chromatin of the egg can scarcely be said to be in a network, but rather to consist of strands which are more or less united (figs. 17, 18), that of the male nucleus being much coarser than that of the polar nuclei. When the sperm reaches the middle of the sac, it still has its curved or vermiform shape, while the contour of the polar nuclei may vary, sometimes being quite curved before coming together (fig. 17), but at other times only changing to this shape as pressure is exerted by contact. The three nuclei upon uniting may be variously twisted about each other, the male nucleus usually twisting more than the others and recognizable by

merely an undifferentiated tissue which practically continues the tissue of the gametophyte, that is, it is simply growth and not organization."

From 1902 to 1913 practically nothing new was published on the subject of fertilization. In 1913 BLACKMAN and WELSFORD (1) reported that the chromatin of the vermiform male nucleus was in a network, although not the network of a resting nucleus, this condition becoming more noticeable later on. At times they also noted that the chromatin of the egg might become threadlike just previous to fusion.

The most recent paper on fertilization is by SAX (**16**) in 1916 on fertilization in *Fritillaria pudica*, in which he noted that the vermiform sperm lay indented in the egg for some time before the membranes between them disappeared. The chromatin was in more or less of a network and the granules were of various sizes. When the membranes at the surface of contact broke down, the contents of the two nuclei mingled and were not distinguishable from each other. The spirem usually appears after this. Triple fusion was also complete and the resulting nucleus divided before that of the fertilized egg.

In none of these cases have the chromatin changes been carefully followed from the time of contact of the nuclei until the completion of the first division, the emphasis previously having been placed upon the actual coming together, the uniting, and the very earliest steps in division.

The process of fertilization and distribution of the chromatin contributed by the egg and sperm in *Pinus* and *Abies* has been carefully worked out by FERGUSON (**4, 5**) and HUTCHINSON (**12**), and it was with the desire that something of this nature should be done for angiosperms that the present investigation was undertaken.

Materials and methods

For fertilization of the egg, *Trillium grandiflorum* was used, the material being collected in damp woods along the Des Plaines River, northwest of Evanston, Illinois, from May 3 to May 26, 1916. The first collection was made at the time of pollination, although the pollen tube was not seen in the micropyle until two weeks later,

while in the collection of May 26 dividing endosperm nuclei and second division of the fertilized egg were found for the first time. *Lilium Martagon*, collected from the garden of Indiana University, Bloomington, Indiana, in May 1916, 96 and 120 hours after pollination, was more favorable for the first division of the endosperm nucleus. The former material was killed and fixed in chrom-osmic-acetic acid 1–2 hours and then in chromo-acetic acid 24–36 hours, washed, dehydrated, and imbedded either from chloroform or xylol. *Lilium Martagon* ovaries were killed and fixed in chrom-osmic-acetic acid 24–36 hours, washed, dehydrated, and imbedded from chloroform. All sections were cut 12 μ thick and both modified triple and Heidenhain's iron-alum-haematoxylin were used for staining, the latter being more satisfactory for most stages, as the chromatin was more sharply differentiated.

Formation of the primary endosperm nucleus

For the development of the spirem and the first division of the endosperm nucleus, *Lilium Martagon* was found to be very favorable, as many dividing primary endosperm nuclei were found in the sacs of material killed 96 and 120 hours after pollination. Activity did not cease at the end of the first divisions, for as many as 12–16 nuclei were found in many sacs of the older material (fig. 29).

The sperm comes in contact with the polar nuclei before these two have fused, although they may be in contact or in close proximity (fig. 17). These three nuclei will usually be found in the center of the sac where just previous to the triple fusion the two polar nuclei were to be seen.

The chromatin of the egg can scarcely be said to be in a network, but rather to consist of strands which are more or less united (figs. 17, 18), that of the male nucleus being much coarser than that of the polar nuclei. When the sperm reaches the middle of the sac, it still has its curved or vermiform shape, while the contour of the polar nuclei may vary, sometimes being quite curved before coming together (fig. 17), but at other times only changing to this shape as pressure is exerted by contact. The three nuclei upon uniting may be variously twisted about each other, the male nucleus usually twisting more than the others and recognizable by

its coarser chromatin strands (fig. 18). In fig. 18 the three nuclei as a whole present a more or less globular contour, although the nuclear membranes are still present at the surfaces of contact.

In *Trillium grandiflorum* the three nuclei, which unite to form the primary endosperm nucleus, are all alike in shape, it being impossible to distinguish the male nucleus from the two polar nuclei by its form or size (fig. 16). Since the mass of the three nuclei is so large, it is often impossible to find parts of all three nuclei in one section, and frequently only two will be visible (fig. 19). All three contain nucleoli, sometimes one, while at other times there are many. The chromatin strands thicken until they may be traced for a considerable distance (figs. 19, 20). While in some instances the membranes still separate the nuclei (fig. 20), at other times they are not visible, as in fig. 19; but, nevertheless, where the chromatin contributed by one nucleus leaves off and that of another begins is very easily seen.

Up to the period when the spirem has assumed its mature thickness, the separating membranes may not have entirely disappeared (fig. 21), and in some cases the three groups of spirems are plainly evident. From fig. 22 it could be concluded that a complete fusion or intermingling of chromatin had previously occurred, but such has not happened, for in the next section of this same primary nucleus parts of all three nuclei are seen (fig. 21). Even at this stage, before the complete breaking down of the separating membranes, segmentation has begun and spindle fibers are forming about the group. As far back as the coming together of the two polar nuclei and the sperm nucleus, a surrounding complex of fibers could be seen (fig. 18).

In fig. 21 the fibers have commenced to radiate out into the cytoplasm, followed after a short period by a complete segmentation of the spirems, resulting in three groups of chromosomes being scattered upon the three arms of the tripolar spindle, respectively (figs. 23, 24). As the tripolar structure gradually assumes the form of a bipolar spindle, the chromosomes, which were previously lying upon the third arm, are pulled into line with the other two groups, thereby forming a typical bipolar spindle (figs. 24, 25). The chromosomes now thicken and are typically arranged into the

equatorial plate of the bipolar spindle; but even yet the third group of chromosomes is recognizable, as can be seen at the left in the group in fig. 25.

After this stage the chromosomes contributed by each of the polar nuclei and by the sperm nucleus are no longer distinguishable (fig. 26). No trace of such distinction is seen in early metaphase or later spindle phases. How the various chromosomes finally arrange themselves upon the spindle and their distribution could not be ascertained in this investigation. When the $3x$ chromosomes have gathered upon the equatorial plate of the bipolar spindle, each very much elongated chromosome splits longitudinally (fig. 26) preparatory to a typical equational division. No intermediate stages between early metaphase and early telophase were found. As the chromosomes reach the poles, they are somewhat shorter than when leaving the equator, and from the count, as seen in fig. 27, $3x$ chromosomes have passed to each pole.

In the third division of the endosperm nuclei of *Trillium grandiflorum* a peculiarity was noted. In one of the dividing nuclei there were still to be seen the three groups of chromosomes upon the spindle, each group consisting approximately of six chromosomes, or the haploid number. It is easily seen that there is a great similarity in appearance between this third division of the endosperm and the first division of the primary endosperm nucleus. A similar stage was observed in the second division of the endosperm nucleus of *Lilium Martagon* (fig. 29), showing in the upper dividing nucleus an appearance very similar to that seen in fig. 24. It was not determined how long endosperm division would continue in *Lilium Martagon*, as nothing older than 120 hours after pollination was collected.

Fertilization of the egg and its first division

Trillium grandiflorum furnished the best material for this phase of the investigation, as the later stages of the first division of the fertilized egg were not to be found in *Lilium Martagon* collected 120 hours after pollination.

In *L. Martagon* the chromatin of the egg and the sperm, at the time when the male nucleus lies coiled upon the egg, is similar in

appearance to that described for the polar nuclei and the second sperm nucleus. The chromatin is in strands, that of the sperm being heavier than that of the egg (fig. 1). The sperm fertilizing the egg is very much smaller than the sperm uniting with the polar nuclei at the time of contact and not so vermiform (compare figs. 1 and 17). Fig. 2 illustrates a typical fertilized egg of *Trillium grandiflorum* just a little later in development than that of *Lilium* (fig. 1). In this later stage the chromatin is lumpy, the particles being larger in the sperm than in the egg, and the membranes separating the two nuclei are becoming very thin, so that it is difficult to distinguish them at all times. After this time these membranes are rarely to be found, although in some instances they persist for a longer period (fig. 4).

The chromatin gradually collects into larger groups, forming more or less broken threads connected with each other by fine anastomoses (figs. 3, 4). In many portions of the nucleus of this fertilized egg the parallel nature of some of these strands is quite conspicuous (figs. 3, 4). In some fertilized eggs, as for example in fig. 5, a more or less beaded, although discontinuous, spirem was noted. Even though the nuclear membranes which separated the egg and the sperm have disappeared, the chromatin that has been contributed by each of the two nuclei remains distinct (fig. 3). This condition is much more evident in some fertilized eggs than in others. The sperm at the period of union contains a much smaller amount of chromatin than the egg and throughout most of the subsequent stages this condition persists (figs. 3, 4, 9, 10, 12). During all this time the fertilized egg is growing in size and increasing the amount of chromatin. When the continuous spirem is first formed, it is quite thin (fig. 6), but as the prophase advances the chromatin thread thickens and shortens until a comparatively thick spirem results (figs. 6–12). Instead of one continuous spirem, two distinct spirems are usually to be seen within the single nuclear cavity, although located in different parts of the cavity (figs. 8–10). In some sections such differentiation is not visible (figs. 6, 7). The dotted lines *a–b* in figs. 9 and 10 separate the two spirems, one of which was contributed by the sperm, the other by the egg.

In spite of the fact that a nucleolus is not seen in the sperm when it unites with the egg, very small ones are found in later

stages (fig. 3), and still later, in the spirem stage, a large nucleolus is frequently observed (fig. 9); but at the beginning of segmentation all traces of these nucleoli have vanished.

With the beginning of segmentation, the chromatin threads appear to contract, presenting the appearance of "the second contraction" of heterotypic mitosis (figs. 11, 12). Following this, the nuclear membrane surrounding the two groups disappears, leaving the massed segments lying free in the cytoplasm. Even now the two sets of chromosomes are separate (fig. 12), and to all appearances a spindle is formed about one group of chromosomes and the other set is pulled into the bipolar spindle, for, as late as in fig. 13, the chromosomes contributed by the sperm are distinct from those contributed by the egg. In each of the two groups (fig. 13) there are approximately six chromosomes, or the haploid number. The writer was unable to determine the arrangement of these two sets upon the equatorial plate, owing to lack of material for later stages. From the number of chromosomes seen at telophase and later divisions, each splits longitudinally at metaphase, so that twelve, the diploid number, pass to each pole.

Discussion

A very full, detailed account of fertilization in *Pinus* by FERGUSON and in *Abies* by HUTCHINSON has been published; but a similar account is not to be found for angiosperms.

FERGUSON (**5**) reports that in *Pinus* the chromatin of the egg is arranged in an interrupted reticulum, the network consisting of granules of various sizes in a colorless linin. When the contents of the pollen tube have been discharged into the egg, one of the male nuclei takes up a position on the concave side of the egg, this depression having been formed at the approach of the male nucleus. Gradually from each nucleus a spirem is formed from the respective chromatin material, at which period fibers arise in the region of the spirems and the nuclear membrane gradually fades away. At segmentation these two spirems give rise to two groups of chromosomes, but as they collect on the spindle this distinction is lost. Each chromosome splits longitudinally and each daughter nucleus receives the diploid number. When these daughter nuclei are preparing for second division, the chromatin collects into two

spirems, the steps being very similar to those of the first division, and it is concluded that in all probability they come from the maternal and paternal source respectively, in spite of the fact that in the formation of the daughter nucleus the chromatin has appeared completely fused. Since the subsequent divisions were not followed, it could not be determined how long this dual nature persisted.

The account of fertilization in *Abies balsamea* by HUTCHINSON (12) varies somewhat from that of FERGUSON. The contents of the male nucleus pass into the nucleus of the egg, although the chromatin groups remain distinct, and later, when the two sets of spindle fibers are formed, two sets of chromosomes arise from the respective nuclei. These two spindle complexes unite and the chromosomes of the maternal parent pair with the chromosomes of the paternal parent, after which the fibers disappear. The members of each pair twist about each other, bend, and become transversely segmented at the bend so that there are $2x$ pairs in the fertilized egg. When the second set of fibers appears, the members of the pairs resulting from the transverse segmentation separate for the opposite poles.

HEATLEY (11) has described the development of the embryo sac of *Trillium cernuum*, in which the sac arises from the chalazal daughter nucleus of the megaspore mother cell, two megaspores only being functional. Each functioning megaspore divides twice to form the typically arranged 8-nucleate embryo sac.

In the present study it was not considered necessary to work out the development of the sac, and furthermore, no attempt has been made to determine the method of entrance of the sperms into the sac or their passage to the egg and polar nuclei. BLACKMAN and WELSFORD (1), ERNST (3), GUIGNARD (6, 9, 10), MOTTIER (13, 14), SAX (16), and STRASBURGER (17, 18) have reported on the earlier phase of fertilization, and on the whole have agreed. These same authors have also described in detail the coming together of the nuclei, their chromatin condition, and the breaking down of the nuclear membranes separating them, although GUIGNARD (7–9) differs somewhat from the others on the latter point, which will be spoken of later. By comparing these investigations with those of *Pinus* (FERGUSON 4, 5) and *Abies* (HUTCHINSON 12), it is apparent

to the writer that the present knowledge of certain phases of fertilization in angiosperms is very scanty, especially as to the fate of the maternal and paternal chromatin.

ERNST (3) reports for *Trillium grandiflorum*, and a similar conclusion is reached by various authors for certain other plants, that there is a fusion of the polar nuclei previous to the entrance of the pollen tube; but in not a single case in either *Trillium* or *Lilium* has such a condition been found to exist, the polar nuclei always being distinctly separate, although usually in contact (figs. 16–19). Only a few cases of triple fusion were observed in *Trillium grandiflorum*, although all that were found appeared as illustrated in fig. 16.

BLACKMAN and WELSFORD (1), GUIGNARD (7–9), MOTTIER (14), and SAX (16) have noted that the male nucleus can be distinguished from the egg and from the polar nuclei both by its shape and the condition of the chromatin, since this substance is coarser in the male nucleus, and at times assumes almost a spirem condition previous to fusion. The sperms have been found not always to retain their S or curved form, for in *Trillium* the male nucleus could not be distinguished from the polar nuclei, either by its size or countour (fig. 16).

The three nuclei (superior and inferior polar nuclei and male nucleus) of *Lilium Martagon* become very much twisted about each other very soon after coming in contact (fig. 18), and even previous to this the polar nuclei may have lost their globular form (fig. 17), although the writer failed to find any mention of this in previous accounts. The fibers appear early about the nuclear complex of *Lilium* and gradually merge into the cytoplasm, as FERGUSON (4, 5) has reported for the fertilized egg of *Pinus*. The chromatin is in fine strands and not in a network, as GUIGNARD and MOTTIER have stated. The number of nucleoli in each nucleus may vary from one to several, and in some specimens there are none. As SAX (16) has said for *Fritillaria*, at the time of contact the chromatin is threadlike, with large irregular pieces of chromatin scattered throughout.

In many instances the separating nuclear membranes are still to be seen when the chromatin has been transformed into a

comparatively heavy spirem (fig. 20), and in some instances at the beginning of segmentation fragments of it still remain (fig. 21). In cases where the separating nuclear membranes do disappear early, the limits of the nuclei are readily followed (fig. 19). GUIGNARD (**7**), in his first report of double fecundation, says that the chromatin of the sperm enters into more or less of a spirem before fusion with the two polar nuclei, after which, at times, he is still able to recognize the triple origin of the secondary nucleus of the sac. None of his drawings are later than fig. 18 of the writer, and apparently the chromatin is in the same condition. In a later paper (**8**) he describes a similar condition in *Narcissus*.

In *Fritillaria*, SAX (**16**), after stating that the chromatin of the male nucleus frequently passes into a spirem previous to the breaking down of the separating membranes, and in some few instances observing the beginning spirem in the polar nuclei, concludes that there is a complete fusion of the chromatin contributed by the three nuclei, and that this is further proved by finding no incomplete fusions in later stages.

In not a single specimen showing the formation of the primary endosperm nucleus was the writer unable to distinguish between the chromatin of the various nuclei that have contributed to this nuclear complex. From the view obtained in fig. 22 it could readily be concluded that a complete intermingling of chromatin has previously occurred, but when the next section of the same primary endosperm nucleus is examined (fig. 21), such a conclusion is seen to be groundless.

Whether or not the separating nuclear membranes have entirely broken down by the time of segmentation, the spirems remain distinct, and, following segmentation, three groups of chromosomes collect upon the three arms of the tripolar spindle (figs. 23, 24). In none of the literature examined has such a stage been shown or reported, for, if such had, the idea of complete fusion or intermingling of chromatin material could not have been adhered to up to the present time.

Since the chromosomes are very long and quite numerous (36), the writer was unable to follow definitely their final arrangement upon the bipolar spindle. From the appearance of fig. 25 it seems

that the group on the right side of the equatorial plate might be the group that has been pulled into line. Soon after this each chromosome splits longitudinally, as FERGUSON (**4, 5**) has reported for *Pinus*, and all trace of the individuality of the groups is lost for a time.

It has been generally understood that in *Lilium Martagon* fusion and subsequent divisions did not occur unless the top was cut off from the bulb; but in the plants used in this investigation, in which this was not done, the ovaries showed many dividing endosperm nuclei and the sacs were in good condition (fig. 29). In *Trillium grandiflorum* in a sac of four dividing endosperm nuclei (fig. 28), and in *Lilium Martagon* in a sac of two dividing nuclei (fig. 29), as described previously, three groups of chromosomes are seen on the spindle. This corresponds to the condition of the second division of the oosphere, as FERGUSON (**4, 5**) has reported for *Pinus*, in which she notes that the second division is like the first, there being two spirems.

Figs. 28 and 29 distinctly show the three groups, and if such a condition is normal the question arises whether the male and female chromatin remaining distinct is the cause of the mottled appearance of some hybrid endosperms as found in *Zea Mays*. As has been observed by many investigators upon chromosome count in endosperm when it consists of many nuclei, the number varies in the different nuclei, there no longer being the $3x$ number. If in some of the divisions, when there is not an equal distribution of chromosomes, which is common in endosperm divisions, one group should pass to one pole and two to the other, the chromatin brought in by the sperm would then be in one nucleus by itself, or with one of the polar nuclei, thus causing the mottled appearance in the endosperm as seen in *Zea Mays*.

The earlier writers on double fertilization, STRASBURGER (**17, 18**), MOTTIER (**14**), NAWASCHIN (**15**), and ERNST (**3**), and the latest investigator SAX (**16**), concluded that there was an intermingling or a complete fusion of the chromatin contributed by the sperm and two polar nuclei in the formation of the primary endosperm nucleus, and ERNST (**3**) further stated that he was unable to recognize at segmentation or in spirem the chromatin

that had been contributed by the respective nuclei; but the writer, because of the numerous specimens showing the distinct spirems, the three groups of segments, and the groups on the tripolar spindle, is unable to accept these conclusions for the primary endosperm nucleus of *Trillium grandiflorum* and *Lilium Martagon*.

What has been said for the chromatin of the primary endosperm nucleus of *Lilium Martagon* applies for the most part to that of the fertilized egg of *Trillium grandiflorum* and the early stages of *Lilium Martagon* and *L. philadelphicum*.

After a careful investigation of *L. Martagon* and *L. candidum*, MOTTIER (**13**) reported in 1898 that there is a complete fusion of the male nucleus and the egg nucleus in the resting condition, the chromatin being in a fine network. If the subsequent steps are not followed out, such an interpretation could be made for *Trillium*. Figs. 1 and 2 show fertilized eggs in which the sperm is lying coiled upon the egg, the male chromatin material being in coarser strands than in the egg. In some instances the nuclear membranes separating the nuclei break down early (fig. 3), while in others they persist for some time (fig. 4). It was the appearance of such stages as fig. 3, and some that will be spoken of later, that caused previous investigators (MOTTIER, NAWASCHIN, SAX, STRASBURGER, and others) to conclude that there was a fusion to the extent that the individual components were not recognized.

From sections showing the spirems (figs. 6–11) it appears at first sight that the interpretations of figs. 9 and 10 would be different from those of figs. 6 and 7; for in figs. 9 and 10 two spirems stand out distinctly, while in the other two there appears to be only one. If the reader will consider all the various angles from which the fertilized egg might be cut and all the various positions the two spirems might occupy within the cavity, it will be apparent that frequently the sections might be so cut that the dual nature of the spirems would not be seen. The significance of the contraction, similar to the second contraction of the heterotypic mitosis that occurs just previous to or during segmentation (figs. 11, 12), the writer is unable to interpret. There was no tripolar spindle observed in the fertilized egg, and from the appearance of fig. 13 it seems that only a bipolar spindle is formed and the second group

of chromosomes is pulled in upon it. In each group there are approximately six, the haploid number.

In 1891 GUIGNARD (**6**) pointed out in *Lilium Martagon* that there were two spirems and that one-half of the chromosomes on the spindle were contributed by the male parent and one-half by the female parent. No drawings were made to substantiate these views, and ERNST (**3**) and MOTTIER (**13**) apparently made it so conclusive that there was a complete fusion or intermingling of chromatin that GUIGNARD's earlier views were discarded and practically forgotten. In later papers GUIGNARD himself did not place much emphasis upon these earlier views.

SAX (**16**) stated that a spirem was frequently found in the egg and sperm before fusion occurred (fig. 21), but says "the rare appearance of such cases as that of the spirem stage in the egg and male nuclei when their outlines are still distinct, is probably of little significance in this respect. It is probable that these nuclei subsequently fuse completely, because no later stage of incomplete fusion was found."

Many writers have looked upon the number of nucleoli present in the fertilized egg as an indication that fertilization has or has not occurred. This, as ERNST (**3**) has pointed out, is not a safe indicator, for, as shown in figs. 5, 6, 9, 19–23, the nuclei have already united and from two to many nucleoli are present.

SAX (**16**) says "fig. 19 shows a stage where the common boundary has disappeared, the contents apparently mingled, and those from the male and female nuclei are not to be distinguished." In case of the formation of the fertilized egg, as in the formation of the primary endosperm nucleus, the writer is unable to agree with SAX and the earlier writers that there is a complete fusion or intermingling of the chromatin of the egg and the sperm; the material so plainly shows that the two remain separate from the time of coming together until the formation of the daughter nuclei. What happens after that does not come within the scope of this paper.

Conclusions

From the stages that have been found in the fertilized egg leading up to the first division, and in the primary endosperm nucleus up to

the time of the third division of the endosperm, it is evident that they are analogous to those of *Pinus*, as reported by FERGUSON (**4, 5**), although nothing was observed that would correspond to those steps in fertilization, as reported by HUTCHINSON (**12**) for *Abies*, which differed from those of *Pinus*.

To the writer the finding of the separate, distinct spirems and the separate groups of chromosomes is added evidence that the chromosomes maintain their individuality from one generation to the next.

In conclusion the writer wishes to state that, according to her interpretation of the word "fusion" as used by previous writers, there was meant a mingling of the male and female chromatin, so that all trace of the individuality of chromatin and chromosomes contributed by the respective parents was lost by the time of the first division. In this investigation no such fusion was found, but instead, an entrance of two or three masses of chromatin, as the case might be, into a more or less single nuclear cavity, the chromatin contributed by the respective parents remaining distinct throughout the preparation for the first division. The writer is unable to state whether fusion in the sense of complete intermingling ever occurs after the completion of the first division.

Summary

1. After the male nucleus and two polar nuclei come together, the separating nuclear membranes persist more or less until segmentation.

2. Three distinct spirems are formed in the primary endosperm nucleus.

3. A tripolar spindle, each arm with its group of chromosomes, precedes the formation of the bipolar spindle.

4. The three groups of chromosomes maintain their identity, at least until several divisions have occurred.

5. The nuclear membranes separating the egg and sperm nuclei disappear earlier than in the preceding case, but the two groups of chromatin remain separate.

6. Two distinct spirems, followed by two groups of chromosomes, arise from the maternal and paternal chromatin in the fertilized egg.

7. There is no complete intermingling of chromatin at fertilization.

To Professor D. M. MOTTIER of Indiana University, under whom the greater part of this work was done, I wish to express my appreciation for the helpful criticisms and help given; to Professor C. B. ATWELL and Professor W. WOODBURN of Northwestern University for the courtesies and encouragement given while working in their laboratory; and to Professor C. J. CHAMBERLAIN of the University of Chicago for the suggestion of this problem.

INDIANA UNIVERSITY
BLOOMINGTON, IND.

LITERATURE CITED

1. BLACKMAN, V. H., and WELSFORD, E. J., Fertilization in *Lilium*. Ann. Botany **27**:111–115. 1913.
2. COULTER, JOHN M., The endosperm of angiosperms. BOT. GAZ. **52**:380–386. 1911.
3. ERNST, A., Chromosomen reduction, Entwickelung des Embryosackes, und Befruchtung bei *Paris quadrifolia* und *Trillium grandiflorum*. Flora **91**:1–36. *pls. 1–6*. 1902.
4. FERGUSON, M. C., The development of the egg and the fertilization in *Pinus Strobus*. Ann. Botany **15**:435–479. 1901.
5. ———, Contributions to the knowledge of the life history of *Pinus*, etc. Proc. Wash. Acad. Sci. **6**:1–202. *pls. 1–24*. 1904.
6. GUIGNARD, L., Nouvelles études sur la fécondation. Ann. Sci. Nat. Bot. VII. **14**:163–296. 1891.
7. ———, Sur les antherozöides et la double copulation sexuelle chez les végétaux Angiospermes. Compt. Rend. **128**:864–871. 1899.
8. ———, Nouvelles recherches sur la double fécondation chez les végétaux Angiospermes. Compt. Rend. **131**:153–160. 1900.
9. ———, L'appareil sexuel et la double fécondation dans les *Tulipa*. Ann. Sci. Nat. Bot. VIII. **11**:365–387. 1900.
10. ———, La double fécondation chez les *Crucifères*. Jour. Botanique **16**:361–368. 1902.
11. HEATLEY, MARGARET, A study of the life history of *Trillium cernuum* L. BOT. GAZ. **61**:425–430. 1916.

12. HUTCHINSON, A. H., Fertilization in *Abies balsamea*. BOT. GAZ. **60**:457–473. 1915.
13. MOTTIER, D. M., Über das Verhalten der Kerne bei der Entwickelung des Embryosackes und die Vergänge bei der Befruchtung. Jahrb. Wiss. Bot. **31**:125–158. 1898.
14. ———, Fecundation in plants. Carnegie Inst. Publ. **15**. 1904.
15. NAWASCHIN, S., Neuen Beobachtungen über Befruchtung bei *Fritillaria tenella* und *Lilium Martagon*. Bot. Centralbl. **77**:62. 1899.
16. SAX, KARL, Fertilization in *Fritillaria pudica*. Bull. Torr. Bot. Club **43**:505–523. 1916.
17. STRASBURGER, E., Einige Bemerkungen zur Frage nach der döppelten Befruchtung bei den Angiosperms. Bot. Zeit. **58**:293–316. 1900.
18. ———, Über Befruchtung. Bot. Zeit. **59**:1–8. 1901.

EXPLANATION OF PLATES III–V

All figures were drawn with the aid of an Abbe camera lucida, with Bausch & Lomb 1.9 mm. oil immersion, and ocular 6. The magnification of all figures except fig. 29 is ×1500; fig. 29 ×420. The plates are reduced to two-thirds their original size.

PLATE III

FIG. 1.—Early union of egg and sperm in *Lilium Martagon*.

FIG. 2.—A little later stage in *Trillium grandiflorum*.

FIG. 3.—Early prophase, separating membranes between egg and sperm having disappeared.

FIG. 4.—A little later, but separating membranes still present.

FIG. 5.—An interrupted spirem in fertilized egg, spirem being more or less beaded.

FIG. 6.—Early spirem in fertilized egg.

FIGS. 7, 8.—Development of spirem.

FIGS. 9, 10.—Two spirems in each nucleus, line *a–b* separating chromatin contributed by egg and sperm respectively.

FIG. 11.—Contraction at time of segmentation.

FIG 12.—Later, contraction still evident; chromosomes in two groups, nuclear membrane having disappeared.

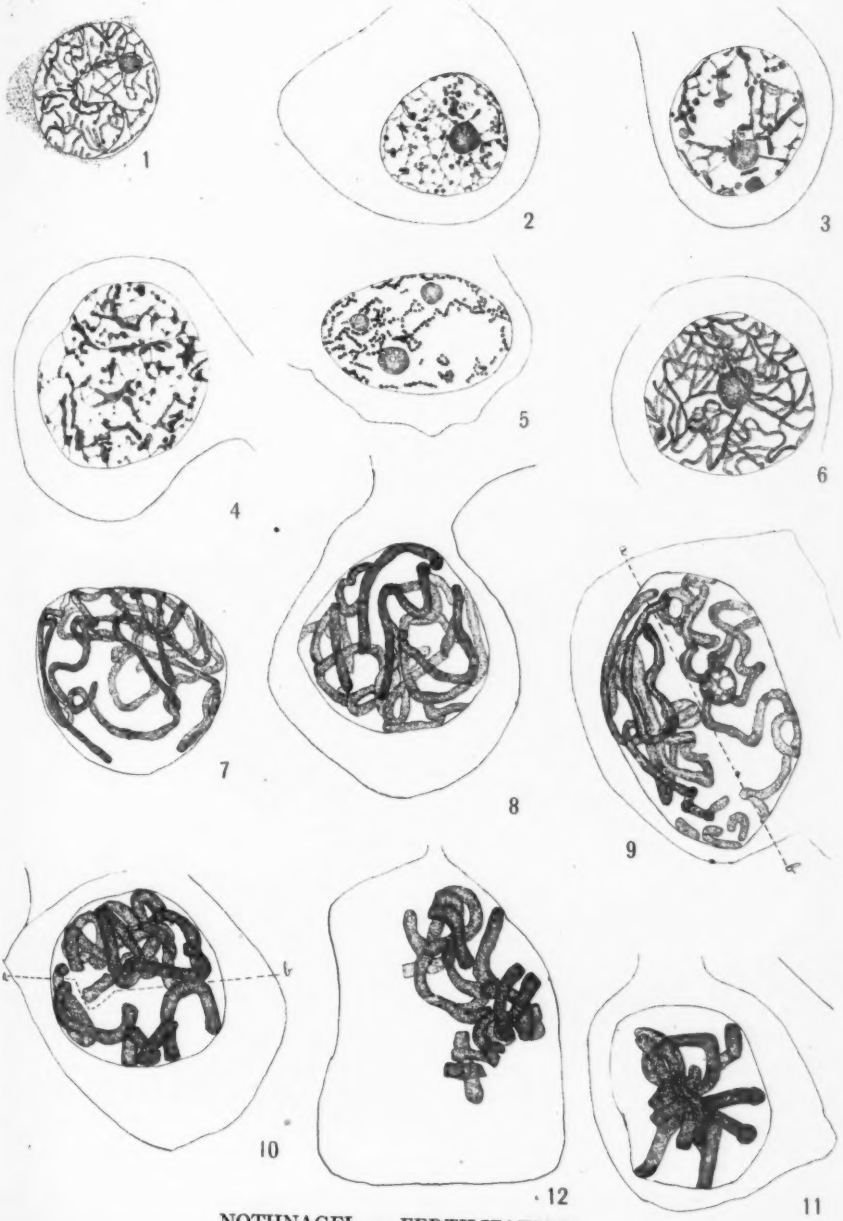
PLATE IV

FIG. 13.—Formation of bipolar spindle, second group being pulled into equatorial plate.

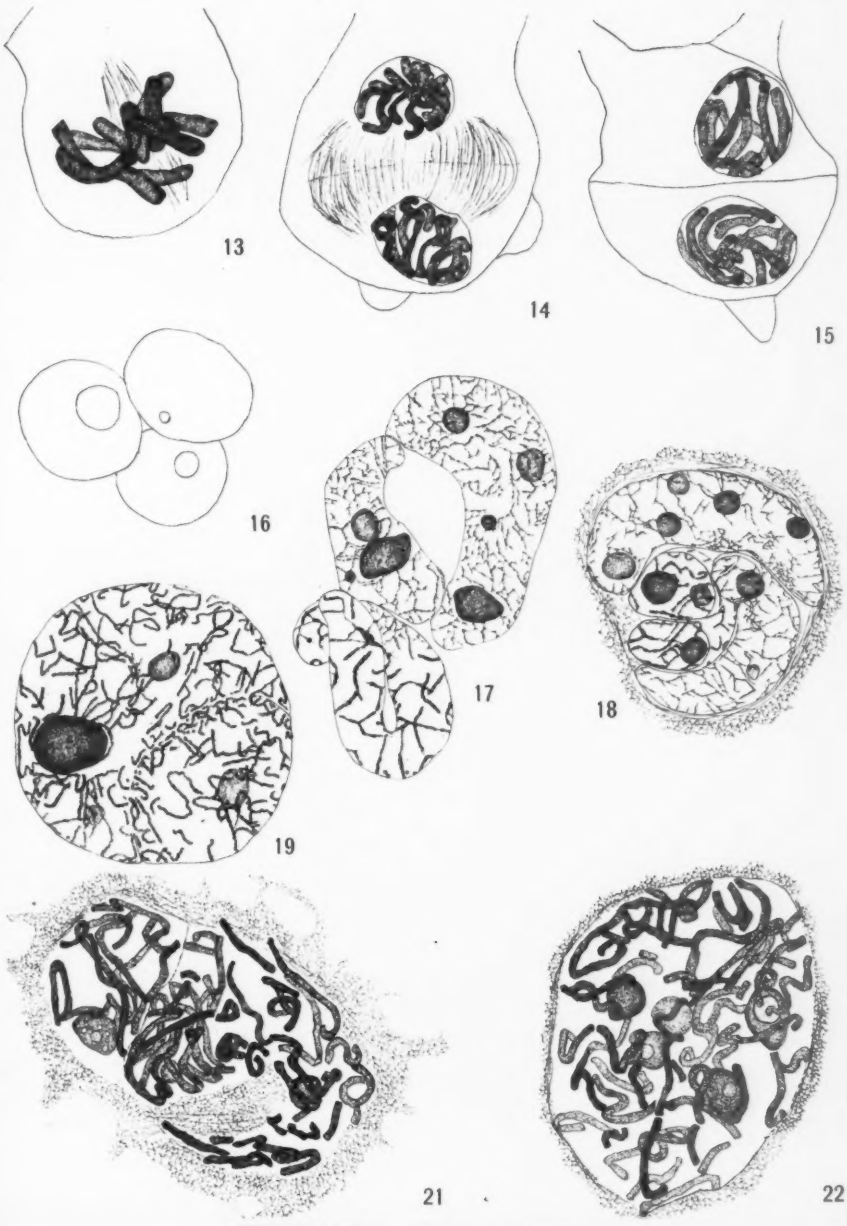
FIG. 14.—Late telophase of first division.

FIG. 15.—Two-celled embryo.

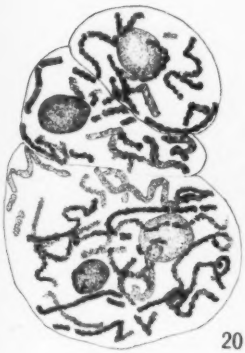
FIG. 16.—*Trillium grandiflorum;* early coming together of three nuclei to form primary endosperm nucleus.



NOTHNAGEL on FERTILIZATION



NOTHNAGEL on FERTILIZATION



20

23

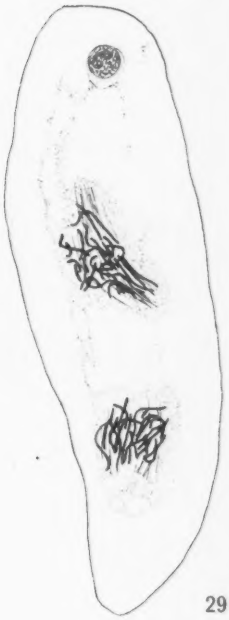
24

25

26

27

28



29

NOTHNAGEL on FERTILIZATION

FIG. 17.—*Lilium Martagon;* same stage as fig. 16, the three nuclei being curved, sperm being more curved and chromatin coarser.

FIG. 18.—Later; fibers are appearing about nuclear complex.

FIG. 19.—Showing two components of primary endosperm nucleus in which the chromatin is collecting in heavier strands and separating nuclear membranes are still evident.

FIG. 21.—Late spirem; separating nuclear membranes still divide the three spirems; fibers beginning to radiate out into cytoplasm.

FIG. 22.—Next section of same primary endosperm nucleus as seen in fig. 21.

PLATE V

FIG. 20.—Formation of spirem, the three nuclei still separate.

FIG. 23.—Three groups of chromosomes upon a tripolar spindle.

FIG. 24.—Slightly later stage; third group is being pulled in with other two groups to form bipolar spindle.

FIG. 25.—Bipolar spindle of first division of primary endosperm nucleus, third group of chromosomes still separate.

FIG. 26.—Slightly later stage; all individuality of groups of chromosomes lost; chromosomes beginning to split longitudinally.

FIG. 27.—Telophase of first division of primary endosperm nucleus.

FIG. 28.—Third division of endosperm nucleus in *Trillium grandiflorum*, showing 3 groups of chromosomes upon bipolar spindle.

FIG. 29.—Second division of endosperm nucleus, upper one showing three groups of chromosomes.

FACTORS DETERMINING CHARACTER AND DISTRIBUTION OF FOOD RESERVE IN WOODY PLANTS

EDMUND W. SINNOTT

(WITH TWO FIGURES)

Introduction

The investigations of RUSSOW, FISCHER, and others upon the character and seasonal changes of the food reserves in woody plants, and the considerable attention which this problem has more recently received, have made us familiar with many of the important facts which it involves; but as to the underlying causes which determine the type of reserve food occurring in any cell and which direct its changes in form and location we are still uncertain. Our present knowledge, derived in greater part from a study of twigs, branches, and small trunks and roots, may be summarized substantially as follows. The major part of the reserves stored up by trees and shrubs during the productive season is evidently composed of starch (fat is also demonstrable, and SABLON (**5**) has emphasized the importance of reserve cellulose as a center of storage). At about the beginning of winter there is a decided reduction in the amount of starch, leading to its disappearance in the phloem and cortex of practically all woody plants in our latitude. At the same time the amount of fat seems to increase greatly. In certain forms, called by FISCHER (**2**) "starch trees," there is no further change, the food reserves in the pith and wood persisting in the form of starch throughout the winter. In others, called by him "fat trees," the starch vanishes in these portions of the stem as well, and fat appears in abundance, constituting the only visible food reserve during the winter. In all woody plants, late winter or early spring sees a regeneration of starch throughout the tissues of the stem and an apparent diminution in the amount of fat. This regenerated starch is used up in the formation of the spring growth, and it is not until summer that a fresh supply begins to be deposited. It has been supposed that at the seasonal changes

starch was converted directly into fat or fat directly into starch, but as microchemical methods have been employed almost entirely this cannot well be proven. The only work involving a quantitative analysis, that of NIKLEWSKI (3), seems to indicate that changes in the two types of reserve food occur independently of each other. It has been observed that the seasonal changes are most marked in twigs and small branches, less so in main stems, and least of all in roots, where fat is scarce and starch persists practically unaltered throughout the winter. The work of FABRICIUS (1) seems to indicate that in the large trunks of spruce conditions may be different from those in small trunks, branches, and twigs, and that starch there may have its maximum in winter and fat its maximum in summer.

That temperature is of importance in producing changes in the character of the food reserves is shown by the fact that starch regeneration may be induced in the winter by bringing twigs from out-of-doors into a warm place. That a subjection to cold during the summer will not cause the characteristic winter changes, however, and that these changes will nevertheless occur in the fall, even though the plants remain under a warm environment, indicate that factors other than temperature must be operative.

The present paper is an attempt to throw light on this general problem by a careful anatomical study of the storage regions of woody plants with a view to determining the exact distribution of starch and fat there and its change from season to season. It contains the results of nearly three years' observations on about 300 species of trees and shrubs belonging to over 100 genera and including all the common species of the northeastern United States, together with many exotic ones in the collections of the Arnold Arboretum. With the exception of a little received from the southern states, all the material studied was gathered in Massachusetts and Connecticut. Special attention was paid to conditions in twigs and young branches, where seasonal changes are most marked. Thin sections of freshly gathered material were cut on the microtome and treated with iodine and Sudan III to bring out the starch and the fat, respectively.

Observations

The results of previous workers as to seasonal changes were substantially confirmed. Although fat is evidently most abundant in the winter months, it is by no means absent during the summer, but at that time it is apt to be masked by the starch. Microchemical evidence as to the relative abundance of either starch or fat at different seasons is necessarily unreliable. It is certain, however, that much of the starch which disappears in the fall does not become converted into fat, but changes to glucose or some other non-visible substance, since in many starch trees large numbers of cells are emptied of starch without causing the appearance of fat. The twigs of some trees, notably species of *Catalpa*, are almost emptied of visible food reserves of all sorts during the winter. There are marked differences between species in their ability to produce fat, as indicated by its abundance in the phloem and cortex. This type of food substance seems to be practically absent in species of *Carya* and is very small in amount in *Fraxinus*, *Acer*. *Syringa*, and others. It is particularly abundant in such forms as *Liriodendron*, *Populus*, and *Pinus*. In general, fat is less abundant in the phloem and cortex of starch trees than of fat trees. There are certain exceptions to this rule, however; notably *Liriodendron*, a starch tree, but rich in cortical fat; and the soft birches, fat trees, but poor in cortical fat. Fat was universally found to be more abundant in the phloem than anywhere else in the plant.

The observations of others that seasonal changes are more marked in twigs than in larger branches and trunks was confirmed. This conservatism is apparently still greater in the roots, where starch was found to be practically unreduced in amount during the winter, a fact recorded by PRESTON and PHILLIPS (4). In the root, too, the amount of fat is very much less than in the stem.

FABRICIUS (1) and others have noted the fact that starch trees are predominantly hard-wooded species and fat trees soft-wooded ones. This rule was in general confirmed by the present study, but a number of exceptions were noticed which we shall later find to be significant. The hard pines, for example, are clearly fat trees; and *Liriodendron*, *Magnolia*, *Ailanthus*, and *Platanus*, all soft-wooded, are clearly starch trees.

Two other general relations between anatomy and the character of the food reserve were noted. Species with diffuse-pored woods are usually either fat trees or have an abundance of fat; those with ring porous wood are almost always starch trees. Narrow-rayed species may belong to either category, but broad-rayed types are prevailingly starch trees.

By no means all the species studied could be classed definitely as starch trees or fat trees. The oaks, ashes, and hickories belong clearly to the former category, and the pines and lindens to the latter; but very many species are intermediate in character, possessing both fat and starch in the wood of the stem. In many instances, also, storage material was noted which was neither starch nor fat but seemed somewhat intermediate in character between the two. The outlines of the original starch grains could sometimes roughly be made out, but the starch content of the cell was apparently coalescing into an irregular brownish mass. This was insoluble in ether and stained neither with iodine nor Sudan III. Its bulky, opaque character indicated that it was actually storage material and not merely the cytoplasm of the cell. It was evident chiefly during the winter, occurring frequently in the cortex as well as in the wood, in cells which had been filled with starch. SUROZ (**6**) called attention to the existence of such material, but apparently it has not been noted by others. If it is indeed a stage in the transition from starch to fat, its composition might perhaps throw light on the difficult problem of the chemistry of fat production in the cell.

Table I presents a rough outline of the character of the food reserve in the pith and wood of the stem (twigs and young branches) of the more common trees and shrubs during the midwinter months, dividing them into those where fat predominates, those which possess considerable amounts of both starch and fat, and those in which starch predominates. This classification should not be regarded as rigid, since a considerable variation has been noted in some of the species and genera, but it represents the average condition observed for each. The character of the reserve in phloem and cortex of course is not included in this table.

The most noteworthy facts brought out by these anatomical investigations, however, concern the exact distribution of the reserve foods in the tissues and their changes from season to season. In all

TABLE I

TYPE OF FOOD RESERVE IN PITH AND WOOD OF STEM (TWIGS AND YOUNG BRANCHES) OF VARIOUS WOODY PLANTS DURING MIDWINTER

PREDOMINANTLY FAT	BOTH STARCH AND FAT
Aesculus	Abies
Betula (some species)	Alnus
Catalpa	Betula (some species)
Cornus (some species)	Chamaecyparis
Dirca	Ginkgo
Juglans (some species)	Gordonia
Leitneria	Juglans (some species)
Picea	Populus (some species)
Pinus	Prunus
Populus (most species)	Rhus (most species)
Pseudotsuga	Robinia
Rhus (some species)	Salix
Taxus	Sambucus
Tilia	Viburnum (some species)
Tsuga	
Viburnum (some species)	

PREDOMINANTLY STARCH	
Acer	Itea
Ailanthus	Jamesia
Berberis	Kalmia
Carpinus	Lindera
Carya	Liquidambar
Castanea	Liriodendron
Celtis	Lonicera
Cephalanthus	Magnolia
Cladrastis	Nyssa
Cornus (some species)	Philadelphus
Crataegus	Platanus
Deutzia	Quercus
Diervilla	Rhamnus
Diospyros	Ribes
Elaeagnus	Rosa
Evonymus	Sassafras
Fagus	Styrax
Fraxinus	Symphoricarpos
Gleditsia	Syringa
Hamamelis	Ulmus
Hydrangea	Vitis
Ilex	Xanthoxylum

species starch disappears in the fall almost completely from phloem and cortex, and even in the starch trees it is much reduced in the wood as well. The reduction in the wood takes place first and most extensively in the regions *immediately around the vessels*. In many

species starch is poorly developed here even in summer. It is in these regions, too, that the "transitional" material is very apt to appear. Furthermore, even in typical starch trees the wood parenchyma cells or ray cells which directly adjoin a vessel frequently contain fat; and in species where both starch and fat occur in the wood the fat is conspicuously abundant near the vessels. In the medullary rays of such forms as most of the poplars and willows, for example, fat is found chiefly in those ray cells which touch a vessel and starch in those which adjoin nothing but fibers. This tendency for starch to be absent and fat to be present in the immediate vicinity of the vessels is obvious in all woody plants in the midwinter season, and suggests that the character of the food reserve may be related in some way to the water supply.

Another anatomical feature which is clearly associated with the kind of food stored in a cell is the character of the cell wall. Wherever this is strongly lignified, thick, and provided with few and small pits, starch tends to remain unchanged throughout the winter. When it is thin or provided with many and large pits, starch tends to disappear and fat to be abundant. Thus in the storage cells of phloem and cortex, the walls of which are quite unlignified, starch vanishes early and completely and fat is very common. In the heavily lignified, thick-walled pith cells which occur in so many species starch remains throughout the winter, and in such cells the reserve food is less modified than in almost any other part of the stem. In branch gaps, where such a pith meets the cortex, the line between the starch-containing and the fat-containing cells is absolutely sharp and coincides exactly with the line between the lignified and the unlignified tissue.

A study of the vertical and ray parenchyma of the wood, the chief seats of food storage in the xylem itself, is particularly instructive in this connection, and furnishes us with a definite anatomical distinction between starch trees and fat trees. Where these cells are thick-walled and have few and small pits, starch predominates; where the walls are thin and well pitted, fat predominates. In hard-wooded species (fig. 1), long noted as starch trees, the parenchyma shares certain of the characters of the other wood cells and has thick, well lignified, square-cornered, and small-pitted walls.

In soft-wooded species (fig. 2), on the other hand, well known as being prevailingly fat trees, the vertical and ray parenchyma is thinner-walled and less heavily lignified, and the cells of the rays tend consequently to be irregular in shape, with oblique or bulging end walls, an outline quite different from the prevailingly rigid and rectangular one of starch tree parenchyma. They are well provided with pits or are so thin as not to require pitting.

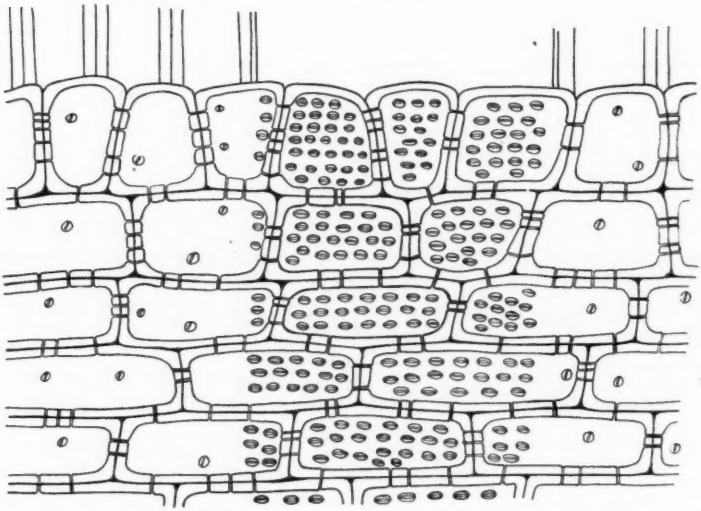


FIG. 1.—*Nyssa sylvatica*, a starch tree: portion of medullary ray of wood seen in radial section as it crosses fibers (at left and right) and a vessel (in center); note thick-walled, squarish ray cells; small pits between ray cells and from ray cells to fibers; and large pits from ray cells to vessel.

Of particular significance are the exceptions already noted to the general rule that there is a connection between hardness of wood and type of food reserve. The hard-wooded pines, for instance, are filled with fat, a circumstance evidently related to the fact that their ray parenchyma and resin canal epithelium (the only seats of storage in the wood) are unlignified and very thin-walled. In *Liriodendron*, *Magnolia*, *Ailanthus*, and *Platanus*, on the other hand, which are soft-wooded but which we have nevertheless observed to contain starch, the rays are made up of thick-walled rectangular

cells precisely like those of starch trees. This type of ray cell is here evidently mechanical in its function, since all these species have wide rays which might collapse or be badly crushed were they not built of strong-walled cells. The vertical parenchyma of these soft-wooded starch trees tends to have thinner walls, and in *Liriodendron*, at least, it contains considerable fat.

On the basis of these facts we are forced to conclude that the hardness of a wood affects the type of food reserve indirectly,

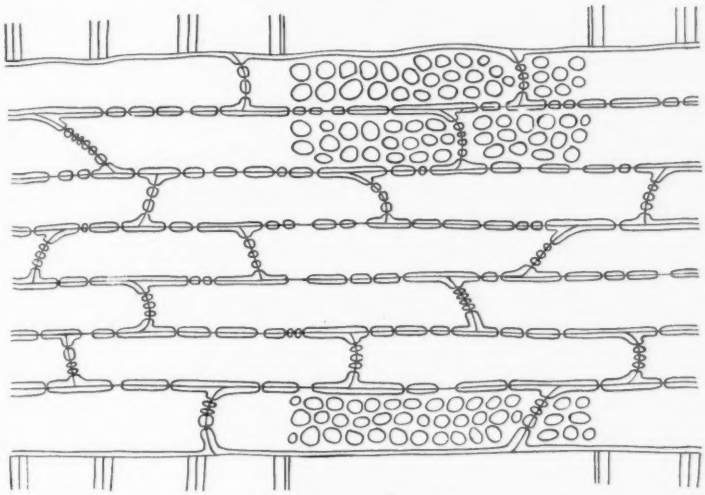


FIG. 2.—*Populus grandidentata*, a fat tree: medullary ray of wood seen in radial section as it crosses fibers (at left and right) and a vessel (in center); note thin-walled ray cells, with slanting or rounded ends, and large pits from ray cell to ray cell and from vessel to (marginal) ray cells.

through its influence on the walls of the storage cells. In certain cases, however, this effect is evidently more direct. The hard-wooded species of *Cornus*, such as *C. florida*, are starch trees, and the soft-wooded ones, such as *C. stolonifera*, are fat trees, although there is no very striking anatomical difference between the parenchyma cells of the two groups. The same fact is noticeable in the hard- and soft-wooded species of *Viburnum* and birches. In these cases it is fair to assume that the high or low degree of lignification

of the conducting and fibrous cells is shared by the parenchyma, and that it is the actual hardness of the wall rather than its structure and pitting which is related to the character of the stored food. We shall later suggest a cause for this relation.

Conditions in starch fibers are of interest here. These are fiber-like, starch-containing cells, and are frequently found in the maples, willows, certain legumes, and other trees. Unlike the parenchyma cells, which are definitely connected with the water supply either by their position next to a vessel or tracheid, or by being linked therewith by other parenchyma cells, these starch fibers usually occur in the midst of non-conducting tissue, being surrounded by fibers of the ordinary type. They possess very small, frequently rudimentary, pits and usually are thick-walled. No instance has been observed by the writer where any reserve food but starch occurs in such cells, and this starch usually stains much more deeply with iodine than that of the ordinary parenchyma, indicating that its water content is lower. The persistent character of the food reserve in these starch fibers is evidently related both to their isolation from channels of water conduction and to their thick and pitless walls.

Discussion

These two main facts which our anatomical survey brings out, (1) that during the winter starch is commonest in regions remote from centers of conduction (both in xylem and phloem), and in cells with thick, well lignified, and small-pitted walls, and (2) that fat is most abundant close to vessels or tracheids, in the phloem, and in cells with thin or unlignified walls and large pits, at once suggest that *the character of the food reserve depends primarily upon the ease with which water or substances carried in water have access to the storage cells*. Where access is slow and difficult, the reserve remains in its summer condition as starch. Where access is easy, it is converted to a greater or less degree into other substances, with the consequent appearance of fat.

In storage cells the walls of which are unlignified, as in the phloem and cortex of all species, and the rays and canal epithelium of *Pinus*, starch is quite absent in the winter and fat is very abun-

dant. There is evidently no impediment to thorough diffusion in such tissues, and conversion may take place far from any center of water conduction.

Where the wall of the storage cell has become heavily lignified, however, even though it is provided with pits (which in such cases are usually very small), diffusion seems to be much impeded, and the reserve food remains unchanged throughout the winter. This is well shown by the terminal cell in the medullary ray of a typical starch tree, which cell, filled with starch and surrounded by its lignified wall, abuts directly upon a starchless, fat-filled cell of the cambial region. There are small pits in the wall between these two cells, but the wall nevertheless seems to serve as an effective barrier between them in preventing rapid diffusion. The same circumstance may often be noted where a ray cell touches a vessel. Here the wall between the two is provided with many large pits (fig. 1), so that communication must be easy. The ray cell in this case is usually without starch in the winter and generally contains some fat. Its neighbors at either end, however, are often full of starch but contain no fat. The tangential ray cell walls, although provided with small pits, seem here also to be permeable with difficulty. This leads us to believe that heavy lignification of the wall is a decided hindrance to the ease of diffusion between cells, and that the pits in such a case are for some reason, perhaps because of their very small size, unable to perform their normal functions.

If the wall of the storage cell is less heavily lignified, or is thinner or more abundantly pitted, entrance of water is evidently easier, and we have noted that in these cases starch is more completely converted and fat is more common. In some instances fat may be limited to the cells directly adjacent to a vessel. In others it may extend to adjoining cells, starch occurring only in the more isolated regions. In these cases fat may be observed extending in from the cambial region along the rays for a considerable distance, thus indicating that diffusion takes place between the cells of the phloem region and the ray cells and affording a marked contrast to conditions at the ends of the rays in starch trees. In the true fat trees diffusion is evidently still more easy, for fat occurs throughout the

rays and parenchyma, even in regions remote from centers of conduction.

In the cases of *Cornus*, *Viburnum*, and others which we have noted, where the degree of lignification of the walls of the storage cells seems to be a factor which determines the character of the food reserve, it probably operates by rendering easy or difficult the diffusion of water into the cell.

That the starch in starch fibers is never converted into fat is evidently due to the fact that there can be little or no communication between them and the water-conducting elements. The willows are illuminating in this connection. Here the phloem, cortex, and medullary rays contain abundant fat in the winter and very little starch, so that the willows have often been regarded as fat trees. In the last annual ring, however, there frequently occur large numbers of starch-filled fibers, so that some writers have included the willows among starch trees. These fibers are almost absolutely pitless. Diffusion of water among them must thus be a slow process, a fact which probably explains this persistence of starch here where it is lost elsewhere in the wood.

This hypothesis, that the character of the food reserve in a cell is dependent primarily upon the ease with which water or substances carried by water can pass from cell to cell, thus makes more understandable the various facts which have been observed as to the type and distribution of reserves in the stems of woody plants from season to season, and is the contribution of anatomy to the problem under discussion. With this as a basis we may allow ourselves to speculate a little as to just what factors are operative in causing the seasonal changes in the food reserve. That change of temperature alone is quite insufficient to account for these is shown by the fact that they take place in plants kept over winter in the greenhouse. The writer has also observed their occurrence in trees growing in the frostless area of the Gulf states. The changes are doubtless due in the last analysis to the action of enzymes, presumably diastase and lipase. There are evidently two quite distinct series of processes, those concerned with changes in starch, and involving the action of diastase, and those concerned with changes in fats, involving the action of lipase.

Two ways at once suggest themselves through which ease or difficulty of diffusion might affect this enzyme activity. First, the water content of the cells may be modified, those to which water has easy access having a higher content than those which are more isolated or protected. That such a condition actually occurs is indicated by the fact that the starch grains in cells near vessels usually stain more lightly with iodine than the others, showing that they possess a higher proportion of water. Differences in water content doubtless affect the whole physiological activity of the cell and may well determine the type of enzyme action. As to why, on this supposition, there should be such radical seasonal alterations, however, is not clear. There are doubtless changes in the water content of the tissues after leaf-fall and again at the spring awakening, and these changes will of course be felt most by those cells to and from which diffusion is easy. In the case of fat, at least, we know that abundance of water favors lipolytic action, and paucity of water favors the synthesis of fat, facts which probably help to determine the increase or decrease of fat with the seasons.

A second suggestion is that the enzymes themselves are carried by the water as it diffuses through the tissues, and thus effect the characteristic changes in the cells which they enter. That these changes take place in the fall we may perhaps ascribe to the presence of enzymes in the sap which is withdrawn into the tissues of the stem from the leaves before the latter are shed. The enzymes would thus be particularly abundant in the phloem, the ordinary channel of conduction from the leaves downward, and they probably would occur in the water of the vessels. They would be progressively less common in those parts of the plant farthest from the leaves. This would explain (1) why the changes are most marked in the phloem and adjacent regions and around the vessels; (2) why they are more marked in twigs than in branches and trunks; and (3) why in roots they are practically absent.

To determine whether or not lipase is actually present in leaves, a series of experiments was undertaken. The leaves of a number of species of trees were gathered in the latter part of summer, dried, and finely pulverized, and the leaf powder of each was mixed with olive oil and bottled up, a number of bottles being made for each

species. The proportion by weight of powder and oil was the same in every case. These were kept at room temperature, and once a week a bottle of each species was taken, the oil was removed from the powder by filtration and was titrated against N/10 sodium hydroxide. The rate of increase of acid measured the strength of the lipolytic action and hence, probably, the amount of lipase. Results showed the ferment to be most abundant in the leaves of those species in which fat was commonest in the stems in winter.

To determine the exact method by which these seasonal changes in the food reserve are effected, however, is beyond the scope of the present paper, the purpose of which is to emphasize the important part evidently played by the minute anatomy of the stem and root in determining the ease of diffusion of water or solutions throughout their tissues and thus affecting the character, distribution, and seasonal alterations in the stored foods, and doubtless in other functional activities of the plant. We may point out that in any such physiological problem as this one a thorough knowledge of the structures concerned is absolutely essential before sound conclusions can be reached.

Summary

1. Previous observations upon the character, distribution, and seasonal changes of the food reserves of woody plants in temperate regions were in general confirmed by the present investigation and were considerably extended.

2. A study of the minute distribution of the food reserves in the tissues of the stem (twigs and young branches) during the winter shows that (1) starch is commonest in regions remote from centers of conduction and in cells with thick, well lignified, small-pitted walls; and (2) fat is most abundant in and near the phloem, close to vessels, and in cells with thin or unlignified walls or large pits.

3. These facts indicate that the character of the food reserve in any cell depends primarily upon the ease with which water or substances carried by water have access to the cell. Where the movement of liquids is apparently slow and difficult, the reserve persists as starch; where such movement is easy, starch disappears at the beginning of winter and fat is produced.

4. This suggests that differences in the type of food reserve may be due to (1) differences in water content of the various storage cells, resulting in modification of enzyme activity, or (2) differences in the ease with which enzymes have effective access to the storage cells.

The writer wishes to thank the authorities of the Agricultural Experiment Stations of Louisiana and of Florida for material from their states, and the authorities of the Arnold Arboretum for specimens from their extensive collections of trees and shrubs. For several helpful suggestions he is also indebted to Professor W. J. V. OSTERHOUT of Harvard University.

CONNECTICUT AGRICULTURAL COLLEGE
STORRS, CONN.

LITERATURE CITED

1. FABRICIUS, L., Untersuchungen über den Starke- und Fettgehalt der Fichte auf den oberbayerischen Hochebene. Naturw. Zeit. Land. u. Forstw. **3**: 137. 1905.
2. FISCHER, A., Beiträge zur Physiologie der Holzgewächse. Pringsheim's Jahrb. **22**:73. 1890.
3. NIKLEWSKI, B., Untersuchungen über die Umwandlung einiger stickstoffreier Reservestoffe wahrend der Winterperiode der Baume. Beih. Bot. Centralbl. **19**:68. 1906.
4. PRESTON, J. F., and PHILLIPS, F. J., Seasonal variation in the food reserve of trees. Forest Quarterly **9**:232. 1911.
5. SABLON, DU, LECLERC, Recherches physiologiques sur les matieres de reserves des arbres. Rev. Gen. Botanique **16**:386. 1904.
6. SUROZ, J. I., Oel als Reservestoff der Baume. Beih. Bot. Centralbl. **1**:1891.

BRIEFER ARTICLES

MODIFIED SAFETY-RAZOR BLADE HOLDER FOR TEMPERATURE CONTROL

(WITH ONE FIGURE)

The apparatus devised in this laboratory for cutting frozen plant material on the rotary microtome[1] has been found useful in the cutting of paraffin sections also, especially when a modification of the familiar safety-razor blade holder is employed. For the control of the temperature of the knife in cutting paraffin sections LAND[2] describes and figures a trough of metal provided with a nipple at either end in which the microtome knife is placed and through which water of the desired temperature is made to flow. The use of a Gillette safety-razor blade in a proper holder is apparently becoming rather general among plant cytologists. Certainly the use of such blades with classes in microtechnique is a great saving of time and energy as compared with attempts either to allow the students to sharpen microtome knives themselves or to provide them with such knives properly sharpened. In addition, with such a class, consistently successful results in cutting can only be obtained if it is possible to regulate the temperature of the knife and, in some cases, of the material also. Even at 10 μ with refractory material in 52° paraffin it is difficult for most students always to obtain a smooth ribbon at ordinary laboratory temperatures unless the knife is kept cool. To meet this latter situation a simple modification of the usual type of safety-razor blade holder has been employed with such success in this laboratory that a brief description of it seems desirable. We have found the original holder made by STRICKLER[3] the most desirable type of a number at present on the market. To such a holder a small brass tube is attached, as shown in fig. 1. This tube has a bore of 4 mm. and is soldered to the outer leaf of the holder, thus in no way interfering with the separation of the leaves when the safety-razor blade is to be inserted. The tube is extended approximately 6 mm. beyond the holder proper at either end to allow the attaching of small rubber tubes.

[1] GARDNER, N. L., A freezing device for the rotary microtome. BOT. GAZ. **63**: 236–238. 1917.

[2] LAND, W. J. G., A method of controlling the temperature of the paraffin block and microtome knife. BOT. GAZ. **57**:520–523. 1914.

[3] CHAMBERLAIN, C. J., Methods in plant histology. Chicago. 1915 (p. 9).

For class use, where very thin sections are not ordinarily required, we have found that the temperature of the knife in such a holder is sufficiently low if tap water is allowed to flow through the tube. A very short time is required for the temperature of the water to be communicated to the knife. A cooling cell such as LAND'S or GARDNER'S also regulated with tap water may be employed in addition, but its use

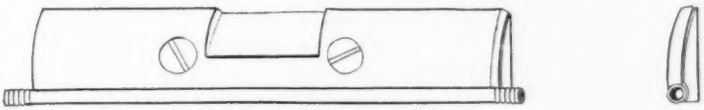


FIG. 1

in most cases is superfluous. Where sections from soft or medium paraffin under 5 μ are required, the modified safety-razor blade holder and the cooling cell are attached to GARDNER'S apparatus with the buckets filled with ice water. Under such conditions sections 2 μ thick have been cut very successfully from a paraffin melting at 53°.—T. H. GOODSPEED, *University of California.*

POLLINATION OF ASCLEPIAS CRYPTOCERAS

Being interested in the mode of pollination of *Asclepias*, I should like to know how PAYSON explains the mode of pollination given in BOT. GAZ. **61**:73. 1916. By a bumblebee's foot I understand the end of the last tarsal joint with two claws and a pulvillus. Does the corpusculum become attached to the foot or to one of these appendages? If the foot is wedged between the anther wings, how does the bee get away without tearing the anther wings, and how does it, or any part of it, enter the cleft of the corpusculum? In pollination, if the bee pulls out its foot with attached corpusculum, what keeps the pollinium from coming out with it? My view of the pollination of *Asclepias*, published in BOT. GAZ. **11**:262–269. 1886, and **20**:110. 1895, is that a single claw, hair, pulvillus, tibial spur, or stump of a retinaculum is caught in the slit between the anther wings and is guided by them into the cleft of the corpusculum. The corpusculum keeps this appendage from again entering the slit. Only one pollinium is caught between the wings and guided into the stigmatic chamber, where it is held so firmly that a pull breaks it loose from the retinaculum. Probably *Asclepias cryptoceras* is a bumblebee flower, but I would not accept the view that it is not occasionally pollinated by other long-tongued bees, or butterflies, unless it is shown that these insects do not have proboscides long enough to reach the nectar.—CHARLES ROBERTSON, *Carlinville, Ill.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Estimation of plant carbohydrates.—In a series of papers from the Rothamstead Experimental Station, DAVIS, DAISH, and SAWYER have reported the results of a critical study of existing methods for the estimation of carbohydrates in plant extracts, and have suggested a number of improvements and modifications designed to secure greater accuracy. In the initial paper of the series, on the estimation of maltose in solution with other sugars, DAVIS and DAISH[1] point out several sources of possible error in the gravimetric method of BROWN, MORRIS, and MILLAR. All samples of asbestos examined, even after previous washing in acid and ignition, contained an easily decomposed silicate, which was rapidly dissolved by the hot alkaline Fehling's solution, thus giving weights which were uniformly too low. Digesting the asbestos for 30 minutes with boiling 20 per cent NaOH and subsequent washing with water removes all material soluble in Fehling's solution, and the authors recommend such digestion as a routine procedure. Since the precipitate of cuprous oxide obtained from plant extracts or from solutions previously subjected to fermentation by yeasts invariably contains copper salts of amino acids and adsorbed colloidal organic matter, the employment of the official method of weighing cuprous oxide as such after drying at 100° introduces an error. This is partially corrected by placing the crucible, after previous washing with alcohol and ether and drying at 100° in a larger crucible and heating for 30 minutes, without the use of a blowpipe, in a powerful flame, subsequently weighing as cupric oxide.

Of the more generally employed volumetric methods subjected to test, the Ling-Rendle method, employing an acid solution of ferrous ammonium sulphate and ammonium thiocyanate as indicator for Fehling's solution, is accurate to at least 0.3 per cent, and is also much more rapid than the Bertrand permanganate method. The latter was found to have an error of 1 per cent with maltose, 1.5 per cent with dextrose, while the results for cane sugar were 3.5 per cent low, as the 2 per cent HCl used for inversion caused considerable decomposition of levulose. In estimating cane sugar in solutions containing maltose, it is impossible to use HCl at 70° for inversion, since maltose is also hydrolyzed, while pentoses undergo decomposition; nor can 2 per cent citric acid be employed, since the use of basic lead acetate to precipitate tannins, amino acids, etc., and the subsequent precipitation of lead with Na_2CO_3,

[1] DAVIS, WILLIAM A., and DAISH, ARTHUR JOHN, A study of the methods of estimation of carbohydrates, especially in plant extracts. I. A new method for the estimation of maltose in presence of other sugars. Jour. Agric. Sci. **5**:437–468. 1913.

leaves in solution a quantity of sodium acetate sufficient to practically inhibit inversion by 2 per cent acid. The authors find that 10 minutes' boiling with 10 per cent citric acid completely hydrolyzes cane sugar without affecting maltose or decomposing pentoses, and recommend this method. Inversion by invertase also gave quantitative results which were too low, apparently, because maltose is carried down in the precipitation with alumina cream. It was impossible to estimate maltose in plant extracts by hydrolysis for 3 hours with dilute boiling HCl or H_2SO_4, as recommended by BROWN and MORRIS, since there was destruction of at least 30 per cent of the levulose present, with measurable amounts of dextrose, by such treatment with any concentration of acid sufficient to effect complete hydrolysis of the maltose present. Hydrolysis with 2.44 per cent HCl at 70° gave no better results; in a 1 per cent solution only 94 per cent of the maltose had been converted after 24 hours' boiling and there had been material destruction of the levulose present. The authors therefore adopted fermentation of the maltose-containing solution with pure cultures of maltose-free yeast as the only satisfactory procedure. The solution is freed of tannins, amino acids, etc., with basic lead acetate, is then made lead-free by adding solid Na_2CO_3, filtering, acidifying, treating with H_2S, and finally making slightly acid to litmus with dilute Na_2CO_3. Three yeasts, *Saccharomyces exiguus*, *S. anomalus*, and *S. marxianus*, were used, the fermentation being continued at 25° for 31 days. All gave good results, but *S. exiguus* is best for general use, since it is least sensitive to acid and its less bulky growth causes less contamination of the cuprous oxide precipitate with salts of amino acids. Checks fermented with ordinary distillery yeasts permit the making of a correction for pentoses remaining after the other sugars have been destroyed. Pentoses were determined by distillation of an aliquot of the solution with HCl at 70° and weighing the furfural as phloroglucide.

The authors' assertion that maltose is hydrolyzed by HCl at 70° has been questioned by KLUYVER,[2] and DAVIS has consequently presented further evidence[3] by reporting the results of a series of experiments with a 1 per cent solution of maltose, carried out under exact Herzfeld conditions, which show a rather uniform loss by hydrolysis of about 2 per cent of the maltose present.

The authors present a scheme for the analysis of plant extracts which may be summarized as follows. The extract is evaporated to small volume in vacuo and made up to 500 cc. Duplicate 20 cc. portions are evaporated to dryness and the drying completed in vacuo for dry matter determinations. The remainder of the solution is treated with basic lead acetate, filtered, and made up to 2000 cc. A portion of this is freed of lead, made up to convenient

[2] KLUYVER, A. J., Biochemische Suikerbepalingen. pp. 223. Boekhandlung E. J. Brill, Leiden. 1914.

[3] DAVIS, WILLIAM A., The hydrolysis of maltose by hydrochloric acid under the Herzfeld conditions of inversion. A reply to A. J. KLUYVER. Jour. Agric. Sci. 6:413–416. 1914.

volume, and divided into two portions. Upon one of these a determination of direct reduction, representing total dextrose, levulose, maltose, and pentoses, is made; the other is employed for determination of cane sugar by inversion with 10 per cent citric acid and with invertase. The remainder of the 2000 cc. of solution is freed of lead and divided into portions. Upon one of these maltose is determined by fermentation with *S. exiguus* or other maltase-free yeast, checked by fermentation with ordinary yeast; the remaining portion is distilled with HCl for the determination of pentoses.

The second paper of the series deals with the methods of estimating starch in plant material.[4] The modified Sachsse method, in which starch is hydrolyzed by boiling HCl, is said to be valueless for two reasons: such plant materials as leaves and seeds contain pentosans and other compounds which are broken down, yielding reducing sugars which are computed as dextrose, while the prolonged boiling with acid destroys some of the dextrose present. O'SULLIVAN's method of estimating starch by converting it into a mixture of dextrin and maltose by the use of ordinary diastase is also shown to give rise to low results; plant material freed of sugar by alcohol extraction still contains tannins, amino acids, and other compounds which necessitate precipitation with basic lead acetate, and a considerable quantity of the dextrin present (15–20 per cent under the conditions of the experiments) is carried down by the lead precipitate and thus lost to the analysis. The authors show that this loss of dextrin is avoided by the use of taka-diastase. When taka-diastase is allowed to act for 6 hours at 38° upon previously gelatinized starch, the whole of the starch is converted into a mixture of maltose and dextrose, continued action resulting in a steady increase in the amount of dextrose, until final equilibrium is attained. The authors therefore adopt the following method. Material for analysis is prepared by dropping the freshly collected leaves or other parts into boiling 95 per cent alcohol to which 1 per cent of concentrated ammonia has been added; immediate destruction of all enzymes is thus assured. Sugars are removed by 18–24 hours' continuous extraction in a special apparatus of the Soxhlet type; the material is freed of alcohol by pressing in a Buchner press and drying 18 hours in a steam oven. It is then ground and bottled for analysis. Samples taken for analysis are dried in vacuo before beginning actual work upon them. As leaf materials usually contain considerable quantities of gum, amylans, and other non-starch constituents which yield reducing sugars, it is necessary to remove these by extraction with a large volume of water for 24 hours at 38°, followed by thorough washing. The material is now boiled with water 30 minutes to gelatinize the starch, cooled to 38°, taka-diastase added (0.1 gm. for 10 gms. vacuum-dried material), and the mixture kept for 24 hours at 38° after the addition of a little toluene. The diastase is then

[4] DAVIS, WILLIAM A., and DAISH, ARTHUR JOHN, Methods of estimating carbohydrates. II. The estimation of starch in plant material. The use of taka-diastase. Jour. Agric. Sci. **6**:152–168. 1914.

destroyed by boiling, the residuum is filtered, washed, the solution made to volume, precipitated with lead, using care to avoid an excess, and portions are taken for polarization and for reduction. Values for the maltose-dextrose mixture are then calculated from the tables of BROWN, MORRIS, and MILLAR.

DAISH[5] has determined the cupric reducing power of xylose and arabinose under the standard conditions prescribed by BROWN, MORRIS, and MILLAR, as all previously published values were determined under somewhat different conditions. He presents tables of the reducing power of each of these sugars for quantities between 10 and 200 mgm. Two curves obtained by plotting the reducing power, expressed as CuO, against the weight of sugar employed are given; from these curves it is possible to determine the weight of sugar corresponding to any given weight of CuO by the employment of a divisor number. The reducing powers of xylose and arabinose are almost identical and differ very little from that of dextrose; thus for 100 mgm. of sugar the divisor number for dextrose is 2.358, for arabinose 2.536, and for xylose 2.490.

DAVIS and SAWYER[6] have presented evidence that free pentoses are quite generally present in the alcoholic extracts of plant material. This evidence they summarize; there are present substances which are soluble in 80 per cent alcohol, which are not precipitable by basic lead acetate, which are not fermentable by ordinary yeasts, and which give the solution reducing power after all fermentable sugars have been destroyed by yeast. This reducing power, if calculated as that of a mixture of xylose and arabinose, agrees almost exactly with the pentose value of the phloroglucide obtained by a KRÖBER-TOLLENS distillation of the solution after previous precipitation with basic lead acetate. These facts can only be explained upon the assumption that the furfural obtained in distillation is derived from free pentoses, not from pentosans, gums, or other sugars.

Various plants, as marigold, turnip, carrot, potato, *Helianthus*, and *Tropaeolum*, showed the presence in the leaves of pentoses in amounts ranging from 0.3 to 1.0 per cent of the total vacuum-dried material, when determinations were made by the KRÖBER-TOLLENS method upon material prepared according to the authors' method. The presence of other sugars, as cane sugar, in the solution to be distilled gives results which are considerably above the true pentose content. Consequently it is necessary, when very accurate determinations of pentoses are desired, to remove the other sugars by fermenting with *Saccharomyces cerevisiae* and to make the determination by distillation of the fermented solution.

---

[5] DAISH, ARTHUR JOHN, Methods of estimation of carbohydrates. III. The cupric reducing power of the pentoses xylose and arabinose. Jour. Agric. Sci. **6**: 255–262. 1914.

[6] DAVIS, WILLIAM A., and SAWYER, GEORGE CONWORTH, The estimation of carbohydrates. IV. The presence of free pentoses in plant extracts and the influence of other sugars on their estimation. Jour. Agric. Sci. **6**:406–412. 1914.

The most recent paper of the series[7] reports the results of an investigation of the generally accepted idea that an excess of basic lead acetate, when added to a solution of invert sugar, precipitates a portion or all of the levulose present as a soluble lead salt. This idea is shown to be incorrect; levulose in dilute solutions is not precipitated by basic lead acetate, even in the presence of chlorides, sulphates, or carbonates. If the acetate be added in excess and allowed to act upon the sugars for a considerable length of time, the amount of levulose present decreases progressively with increase in the time during which the lead is allowed to act. This is due to the formation from the levulose, not of a lead salt, but of a substance having a lower reducing power and much less optical activity than has levulose. It is suggested that this substance may be glutose, which was made by LOBRY DE BRUYN and VAN EKENSTEIN by heating a 20 per cent levulose solution with lead hydroxide at 70–100°, and which was described by them as having about one-half the reducing power of dextrose and as possessing only very slight optical activity. DAVIS considers that basic lead acetate acts at ordinary temperatures in the same way as does lead hydroxide, the action becoming more rapid as the temperature rises.

In order to avoid any loss of levulose when clearing a solution, the basic lead acetate must be added little by little in the cold until precipitation of the impurities is just complete, care being taken that the excess employed is not greater than 1 cc. per 100 cc. of sugar solution (best accomplished by making preliminary tests upon small portions of the filtrate). The solution should at once be filtered through a Buchner funnel, washed, and the excess of lead immediately precipitated by the use of Na_2CO_3 or Na_2SO_4. If excess of Na_2CO_3 be avoided and the solution be shaken up with a little toluene, it may be kept for months without the occurrence of any change. This treatment is very much to be preferred to the use of normal lead acetate, which fails to wholly remove optically active gums and which is a poor clarifying agent, but it is essential to accuracy that the precipitation be conducted in the cold.

The papers here reviewed were preliminary to a series on the formation and translocation of carbohydrates in plants, to be reviewed later.—JOSEPH S. CALDWELL.

Taxonomic notes.—ARTHUR,[8] in continuation of his studies of the Uredineae, has described 23 new North American species in the following genera: *Uromyces* (2), *Puccinia* (8), *Aecidium* (10), *Uredo* (3). The majority of them are from Mexico and Central America.

ASHE[9] has described a new *Vaccinium* (*V. Margarettae*) from the mountains of Georgia and South Carolina, where it occurs in association with *V. vacillans*.

---

[7] DAVIS, WILLIAM A., The estimation of carbohydrates. V. The supposed precipitation of reducing sugars by basic lead acetate. Jour. Agric. Sci. **8**:7–15. 1916.

[8] ARTHUR, J. C., New species of Uredineae. X. Bull. Torr. Bot. Club **45**:141–156. 1918.

[9] ASHE, W. W., Notes on southern woody plants. Torreya **18**:71–74. 1918.

EVANS[10] has described 4 new species of *Lejeunea* from Florida, 2 of which seem to be endemic to that state. Of this group of liverworts Florida is now known to contain 44 species of the 48 recorded from the United States.

FERNALD[11] has described 2 new species of *Rosa* (*R. johannensis* and *R. Williamsii*) from northern Maine and adjacent Canada.

PETCH[12] has described 138 new species of fungi from Ceylon, representing approximately 75 genera. Among them there are 32 new species of *Uredo*.

WIEGAND[13] has published the result of his studies of *Elymus* in Eastern North America, discussing 7 species, one of which (*E. riparius*) is described as new.—J. M. C.

Phylogeny of Filicales.—In continuation of his studies of Filicales, BOWER[14] has presented the Pteroideae. The observed details of phyletic relationships among the genera are too numerous to recite, but the paper contains a wealth of material for the special student. In a former paper of the series BOWER suggested that the leptosporangiate ferns, exclusive of the Osmundaceae, may be grouped into two phyletically distinct series: the Superficiales, in which the origin of the sorus is constantly from the surface of the leaf; and the Marginales, in which it is as constantly from the margin. All of the Pteroideae belong to the Marginales, and they show analogies with the Superficiales, especially in those forms which have apparently superficial sori. He shows that such sori result from "a slide of the marginal sorus to a superficial position." "The Superficiales are believed to represent ferns in which that slide took place so early in their descent that the two sequences must be held to be phyletically distinct, notwithstanding those analogies."—J. M. C.

Atmometry.—The desirability of having an atmometer so constructed as to indicate the magnitude of the atmospheric evaporation power at any given moment is discussed by JOHNSTON and LIVINGSTON.[15] Attempts to produce such an instrument are described, but so far it has not been possible to overcome certain difficulties in converting evaporation power into pressure. The nearest approach to such an instrument which has proved successful is a device

[10] EVANS, A. W., Noteworthy *Lejeuneae* from Florida. Amer. Jour. Bot. **5**: 131–150. *figs. 5*. 1918.

[11] FERNALD, M. L., *Rosa blanda* and its allies of northern Maine and adjacent Canada. Rhodora **20**:90–96. 1918.

[12] PETCH, T., Additions to Ceylon fungi. Ann. Roy. Bot. Gard. Peradeniya **6**:195–256. 1917.

[13] WIEGAND, K. M., Some species and varieties of *Elymus* in Eastern North America. Rhodora **20**:81–90. 1918.

[14] BOWER, F. O., Studies in the phylogeny of the Filicales. VII. The Pteroideae. Ann. Botany **32**:1–68. *figs. 43*. 1918.

[15] JOHNSTON, EARL S., and LIVINGSTON, B. E., Measurement of evaporation rates for short time intervals. Plant World **19**:136–140. 1916.

which permits two readings to be made with a very short time period between. An atmometer cup is mounted over a reservoir from which it may be cut off at will by means of a glass cock. It is also connected with a finely graduated burette from which the water will be drawn when the reservoir cock is closed. A reading can be made in a very short time at any desired intervals, and the average evaporating power for the period of observation can be calculated. Comparison of different environments is easily made.—CHARLES A. SHULL.

Embryo sac of Oenothera.—ISHIKAWA[16] has published a very full account of the behavior of the gametophytes and the fertilization phenomena in *Oenothera nutans* and *O. pycnocarpa*, as well as in their hybrids. These two species were formerly included in *O. biennis*. The embryo sac arises from either the chalazal or micropylar megaspore, and often both develop simultaneously into complete embryo sacs. The sac is tetranucleate, lacking the antipodals and one of the polar nuclei. In fertilization one of the male nuclei fuses with the remaining polar nucleus, resulting in diploid endosperm. Self-sterility of some of the hybrids is due to feeble growth of the pollen tube. Tetranucleate embryo sacs occur also in *Ludwigia*, *Gaura*, *Godetia*, and *Circaea*.—J. M. C.

Iron in nutrient solutions.—CORSON and BAKKE,[17] working upon wheat and Canada field peas, have studied the relative merits of ferrous and ferric phosphates in nutrient solution. They find that iron in the nutrient solution is more important than generally considered; that ferric phosphate is more effective than ferrous phosphate, especially for wheat; and that ferric phosphate in the concentration suggested by SHIVE (0.0044 grams per liter) gives maximum dry weight.—WM. CROCKER.

Polyembryony.—HARVEY,[18] in connection with recording a case of polyembryony in *Quercus alba*, has given a summary of the recorded cases of polyembryony in angiosperms. The list includes 36 cases, scattered through "15 of the 49 alliances." In the case of *Quercus* reported two vigorous embryos occurred in the acorn, and it is of special interest because this is said to be the first reported case of polyembryony "in the first 13 alliances of the Archichlamydeae."—J. M. C.

---

[16] ISHIKAWA, M., Studies on the embryo sac and fertilization in *Oenothera*. Ann. Botany **32**:277–317. *pl. 7. figs. 14.* 1918.

[17] CORSON, G. E., and BAKKE, A. L., The use of iron in nutrient solution for plants. Proc. Iowa Acad. Sci. **24**:477–482. 1917.

[18] HARVEY, LEROY H., Polyembryony in *Quercus alba*. Mich. Acad. Sci. Rep. 1917. 329–331.